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EFFECTS OF CALCIUM DEFICIENCY ON NITRATE ABSORPTION AND ON METABOLISM IN TOMATO[1]

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(WITH THREE FIGURES)

Introduction

This series of experiments was undertaken in an effort to determine the relation of calcium to the metabolism of carbohydrates and of nitrogenous compounds. Calcium is generally regarded as an essential element, and within recent years several workers (**10, 17, 46, 55**) have described a yellowing and a dwarfing of plants that had been grown in a deficiency of calcium, although they disagree as to the precise effect of such deficiency on the plant.

The tomato was used in the present experiments because it is well adapted to nutrient culture and because it has been used in so many experiments by different workers that a considerable knowledge of its nutrient requirements and metabolism is available.

Experimental methods

The experiments were carried on in the greenhouse at New Brunswick, New Jersey, during the early summer of 1930, with tomato plants of the variety Marglobe. On June 17 about 1,500 plants were selected for uniformity from 3,000 plants that had been grown for several weeks in sifted loam soil in four-inch pots. The plants were about 30 cm. in height, and were rather yellowish green in appearance; analysis showed them to be comparatively high in carbohydrates, but low in nitrogen (tables II, III, IV and V). The roots of each plant were washed free of soil, and the two lower leaves were removed; 300 of the plants were then used for initial

[1] The Kjeldahl and mineral determinations were made in the laboratory of C. S. CATHCART, for whose cooperation the authors wish to express their appreciation. They wish also to acknowledge their indebtedness to O. W. DAVIDSON for his help in obtaining photographs of plant material.

analysis, and the remaining 1,200 were transplanted for experimental treatments to washed quartz sand in new ten-inch clay pots, one or two plants to a pot. The pots were set in shallow enamel-ware pans and for the three weeks of the duration of the experiment all of the plants were subjected to nutrient treatments, each pot receiving daily two liters of nutrient solution. Twice a week each culture was thoroughly flushed with distilled water, and fresh nutrient solution was applied immediately.

The 1,200 plants were divided into four series according to the nutrient treatment which they received (see table I). One series was supplied with a complete nutrient solution that had been shown by previous experiments to be well adapted to the growth of tomato plants. The three other series received solutions lacking either calcium or nitrates or both. From time to time some of the plants were shifted from one nutrient treatment to another. The nutrient solutions employed all had a pH of about 4.7 when applied. After the solution had been in contact with the plant roots for 24 hours it was more alkaline, 6.2 to 6.4, but about the same in all series. The iron content of the initial plants or a trace of iron in the salts employed was sufficient for subsequent growth. Ferrous sulphate, however, was applied to some of the cultures in certain experimental trials described on page 617. Conditions of temperature and humidity during the progress of the experiments were suitable for the commercial production of tomatoes. The plants were grown under the seasonal light conditions of the greenhouse with the exception of some that were subjected to a period of continuous darkness at a practically constant temperature of 23° C.

Chemical methods

For macrochemical analyses, the plants were divided into seven fractions as shown in table VI. All terms are self-explanatory, except the term petioles, which in this paper includes also the rachis and the large veins of the leaf. Determinations of nitrogenous and carbohydrate fractions were made with fresh and dried tissues respectively, according to procedure previously described in detail (**41**). Aliquots of dried tissue were employed for mineral analyses (**3**).

Microchemical tests, which were made on fresh plant material, were in general those recommended by ECKERSON (**12, 16**), with modifications that will be described along with the presentation of results.

Results

On June 17, at the start of the various nutrient treatments, all of the plants were somewhat stiff and woody, with yellowish green leaves and a few blossoms. Macrochemical analysis showed that they contained no nitrates and no ammonia. They were very low in all forms of elaborated nitrogen but high in carbohydrates (tables II, III, IV, and V). Micro-

scopic examination of the stem showed a high percentage of very thick-walled mechanical and conductive tissue. Even though the plants were growing very slowly, cambium was active throughout the length of the stem. Starch was observed in large quantities in all parenchymatous tissue, especially in the pith, almost to the tip of the stem.

The initial plants contained the following percentage of calcium computed on a green weight basis; stems 0.13, blades 0.32, petioles 0.10, and roots 0.05. Calcium was present in three different forms: (1) calcium oxalate crystals, in the parenchyma of phloem, cortex, and pith; (2) "uncombined" calcium, *i.e.*, calcium that could be detected microchemically by the usual treatment with oxalic acid, and (3) "combined" calcium, *i.e.* calcium that could be detected microchemically only after treatment of fresh sections with a strong base such as NaOH. The terms "combined" and "uncombined" are purely relative, for, of course, all calcium in a cell is to some extent combined. "Combined" calcium was found in all living cells, not only along the walls but rather uniformly distributed through the protoplasts. "Uncombined" calcium was found throughout the protoplasts of all living cells. In collenchyma and parenchyma cells of the cortex and in parenchyma cells of the vascular cylinder, it occurred also along the walls, suggesting the presence of calcium pectate in the middle lamella. Many of the cells, however, especially of the pith, did not show a reaction which would indicate a middle lamella composed of calcium pectate in either plus- or minus-calcium plants.

Plants which received the complete nutrient solution

Throughout the course of the experiments (June 17–July 9) the plants that received the complete nutrient treatment exhibited a vigorous and

Fig. 1. Tomato plants—July 9, 1930. From left to right, plus-calcium plus-nitrate; minus-calcium plus-nitrate; and minus-calcium minus-nitrate.

apparently healthy growth of tops and roots, as shown in figures 1 and 2. Although these plants received a solution fairly high in concentration of nitrates (table 1), they were not soft nor extremely succulent. This was undoubtedly associated with the fact that the initial plants were hard,

FIG. 2. Roots of tomato plants—July 9, 1930. From left to right, plus-calcium plus-nitrate; minus-calcium plus-nitrate; and minus-calcium minus-nitrate.

high in carbohydrates and low in nitrogen (tables II, III, IV, and V), also that the experiments were run for a period of only three weeks. Dur-

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS. (PARTIAL VOLUME MOLECULAR CONCENTRATIONS OF SALTS USED)*

SOLUTION	$Ca(NO_3)_2$	KNO_3	KH_2PO_4	$MgSO_4$	$CaCl_2$
Plus-Ca plus-NO_3	0.0180	.....	0.0090	0.0090	.....
Minus-Ca plus-NO_3	.....	0.0260	0.0090	0.0090	.....
Minus-Ca minus-NO_3	.....	.....	0.0090	0.0090	.....
Plus-Ca minus-NO_3	.....	.....	0.0090	0.0090	0.0180

* With the exception of the minus-Ca minus-NO_3 solution, each solution has a total osmotic concentration value of 2 atmospheres.

ing that time many fruits were set, and if the plants had been grown for a sufficient number of weeks undoubtedly they would have borne a heavy crop of fruit, as did the few plants which were allowed to remain.

During the progress of the experiments, certain factors remained practically constant in the various series of plants. The pH of the expressed sap of the various plant fractions, such as roots, lower stem, upper stem, stem tip, and blades showed little variation from one series to

another, although the usual (**20**) differences in pH of different plant fractions were observed. Microchemical tests failed to show any consistent outstanding variation in pH of respective tissues, although the usual differences (**13, 41**) were noted in pH of pith, xylem, phloem and cortex.

PLANTS DEFICIENT IN CALCIUM

In contrast to the plants that had received complete nutrient treatment, those that were grown from June 17 to July 9 in a solution lacking calcium (figs. 1 and 2) were stunted in vegetative growth and the stems were stiff and woody. Increase in volume occurred for only a brief period after the transfer from soil to sand on June 17. A few blossoms were formed but practically no fruit was set. The upper leaves were characteristically yellow, not yellowish green, whereas the lower leaves, which were thick and stiff, were still fairly dark green. Shortly after July 9 the stem tips of the calcium-deficient plants died.

FIG. 3. Typical roots from tomato plants grown with the minus-calcium plus-nitrate nutrient treatment.

The leaves of some of the plants low in calcium exhibited a peppery brown spotting, apparently the result of iron toxicity rather than any direct effect of calcium deficiency or, on the other hand, of magnesium toxicity (page 618).

The root systems were short and much branched as shown in figures 2 and 3. Branch roots were short and stubby, and some showed characteristic browning of tips and of parenchyma further back, and sloughing off of some of the outermost cells. During the period of the experiments, however, the roots were alive and developed an abundance of apparently healthy root hairs.

The characteristics just noted were substantiated by macro-analysis and microchemical tests. Tables II and III show that the minus-calcium plants were even higher in carbohydrates than the plants that had received the complete nutrient treatment. Associated with the higher concentration of carbohydrates were thicker cell walls in the recently formed xylem.

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Dry matter	14.35	12.25	14.30	13.55	16.10	12.66
Reducing sugars	7.33	2.36	6.27	3.70	7.19	6.40
Sucrose	8.91	5.25	7.96	6.21	8.33	3.99
Total sugars	16.24	7.61	14.23	9.91	15.52	10.39
Starch and dextrin	23.74	8.92	19.17	10.04	25.73	16.54

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

TABLE III

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Reducing sugars	1.05	0.29	0.90	0.50	1.15	0.81
Sucrose	1.28	0.64	1.14	0.84	1.34	0.51
Total sugars	2.33	0.93	2.04	1.34	2.49	1.32
Starch and dextrin	3.41	1.09	2.74	1.36	4.14	2.09

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

Nitrates were comparatively low in the minus-calcium series, but on the basis of macrochemical determinations there was no significant difference between the calcium-deficient and the complete-nutrient plants in percentage or quality of organic nitrogen (tables IV and V). The quality of protein, however, was undoubtedly very different in the stems of the two lots of plants, as in the cells of the plants of the minus-calcium series,

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Total nitrate-free N	0.553	1.530	1.375	1.609	1.649	1.570
Protein N	0.400	0.930	0.975	0.993	1.019	0.997
Nitrate-free soluble N	0.153	0.600	0.400	0.616	0.630	0.573
Basic N	0.020	0.045	0.044	0.119	0.128	0.130
Amino N	0.119	0.462	0.300	0.385	0.370	0.273
Amide N	trace	0.068	0.048	0.127	0.104	0.125
Ammonia N	none	trace	trace	0.002	0.002	0.003
Other N	0.014	0.025	0.008	−0.017	0.026	0.042
Nitrate N	none	0.040	0.025	0.211	0.086	0.270
Total N	0.553	1.570	1.400	1.820	1.735	1.840

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

TABLE V

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

DATE	JUNE 17	JULY 1		JULY 9		
TREATMENT	INITIAL PLANTS	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	MINUS-Ca DARK*
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Total nitrate-free N	0.079	0.187	0.197	0.218	0.265	0.199
Protein N	0.057	0.114	0.139	0.135	0.164	0.126
Nitrate-free soluble N	0.022	0.073	0.058	0.083	0.101	0.073
Basic N	0.003	0.006	0.006	0.016	0.021	0.016
Amino N	0.017	0.057	0.043	0.052	0.059	0.035
Amide N	trace	0.008	0.007	0.017	0.017	0.016
Ammonia N	none	trace	trace	trace	trace	trace +
Other N	0.002	0.002	0.002	−0.002	0.004	0.005
Nitrate N	none	0.005	0.003	0.028	0.014	0.034
Total N	0.079	0.192	0.200	0.246	0.279	0.233

* After July 1, some of the minus-Ca plants were subjected to continuous darkness.

particularly in the phloem region, but also in other parenchymatous tissue, there were granular inclusions that were not evident in the complete-nutrient plants. These inclusions gave positive protein reactions with all the usual reagents. Furthermore, such inclusions were conspicuous in the stem tips of calcium-deficient plants at the time of death of the meristem and shortly before.

Internally the stubby bulbous roots of the plants grown in the solution deficient in calcium showed differentiation of stele practically down to the embryonic tip, whereas in the faster growing roots of the complete-nutrient plants the rapid division and elongation of cells resulted in a long region of elongation between embryonic tip and zone of maturation. Within the stubby tips the planes of cell division were not irregular, although enlargement of cells was greater laterally than longitudinally. A factor which also contributed to the general appearance of stubbiness of the root system was the development of primordia of numerous branch roots, many of which never developed. Constrictions of the roots were not uncommon and were due to localized injury of outermost layers of cells and failure of these cells to enlarge properly, or to disintegration, or to both.

The externally conspicuous browning of roots of the calcium-deficient plants was almost entirely of parenchymatous and meristematic tissue. This was almost always observable first in the root tip, in the endodermis and in isolated cells of the cortex and stele. Later it occurred in practically all parenchymatous tissue, but did not appear to be present in conductive elements of xylem and phloem. Cellulose walls were chiefly concerned, but the protoplasts sometimes became decidedly granular, and this granular mass, as in the stems, was often a golden brown color and gave positive tests for protein with the usual reagents; the reaction with Millon's reagent was particularly strong.

The plants were tested for calcium from time to time during the course of the experiments. Microscopic examination showed the presence of some calcium oxalate in the base of the stem even at the end of the experiment, long after the plants had begun to show signs of calcium deficiency. The usual microchemical test with oxalic acid gave practically negative results, showing that there was little "uncombined" calcium present. However, an appreciably greater quantity was detected by treatment with oxalic acid after the sections had been treated with NaOH. These results suggest that the considerable quantity of calcium shown by macro-analysis to be present in the lower half of the tops (tables VI and VII), consisted largely of calcium oxalate deposits and of calcium so combined with protein or some other substance that it had to be released by hydrolysis before it could react with oxalic acid.

TABLE VI

MINERAL ANALYSES OF TOMATO PLANTS HARVESTED JULY 9
(RESULTS EXPRESSED AS PERCENTAGE OF DRY MATTER)

ELEMENT	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM		SULPHUR	
SERIES	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Upper blades	1.61	0.17	0.67	0.60	0.80	0.49	2.67	3.65	0.98	0.59
Lower blades	3.84	1.10	0.99	0.85	0.50	0.31	2.67	3.28	1.45	1.01
Upper petioles	1.08	0.11	0.92	0.23	0.73	0.78	6.27	9.64	0.38	0.51
Lower petioles	2.23	0.26	1.64	0.33	0.69	0.51	4.70	10.62	0.49	0.49
Upper stem	0.67	trace	0.69	0.24	0.70	0.68	5.25	6.02	0.33	0.31
Lower stem	0.99	0.53	0.50	0.30	0.41	0.43	2.27	4.39	0.28	0.25
Roots	1.26	0.25	0.46	0.60	2.34	0.72	2.23	3.18	0.75	0.55

TABLE VII

MINERAL ANALYSES OF TOMATO PLANTS HARVESTED JULY 9
(RESULTS EXPRESSED AS PERCENTAGE OF GREEN MATTER)

ELEMENT	CALCIUM		MAGNESIUM		PHOSPHORUS		POTASSIUM		SULPHUR	
SERIES	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca	PLUS-Ca	MINUS-Ca
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Upper blades	0.255	0.028	0.106	0.097	0.127	0.079	0.425	0.592	0.156	0.096
Lower blades	0.595	0.254	0.153	0.197	0.078	0.072	0.414	0.759	0.225	0.234
Upper petioles	0.935	0.010	0.080	0.021	0.063	0.074	0.543	0.909	0.033	0.048
Lower petioles	0.245	0.029	0.180	0.034	0.076	0.052	0.517	1.062	0.054	0.051
Upper stem	0.057	trace	0.059	0.030	0.060	0.085	0.452	0.759	0.028	0.042
Lower stem	0.150	0.087	0.076	0.050	0.062	0.071	0.345	0.724	0.043	0.041
Roots	0.149	0.035	0.054	0.084	0.276	0.101	0.263	0.447	0.089	0.077

Plants deficient in calcium and nitrates

The plants that throughout the experiments received a solution lacking in both calcium and nitrates, were in some respects like those that lacked calcium only. They too increased only slightly in size (even less than the minus-calcium series) and were woody in texture; microchemical tests showed that they too were high in starch and devoid of nitrates. Also, they contained practically no uncombined calcium but did contain a considerable quantity of combined calcium. However, they were not so yellow (fig. 1), and what yellowing occurred was chiefly of the older leaves, and not of the tips as observed above in the minus-calcium series. Moreover, the root systems were without the stubbiness of branch roots noted in the plants that lacked calcium only (fig. 3). This difference in appearance of roots and tops may have been due to the fact that with less vegetative extension there was a higher concentration of calcium per unit volume of plant tissue. It will be recalled that the vegetative growth of the plants of the calcium deficient series occurred for only a brief period after the start of the experiments. At that time all plants contained an abundance of calcium (page 607), were, in effect, plus-calcium plants, and were undoubtedly able to absorb and assimilate nitrates if present in the nutrient solution. On the other hand, in the series deprived of nitrates, no nitrates were available at the time of the initial growth of the other series of plants, when calcium was still available.

In an effort to study the absorption of nitrates, some of these plants deprived of both calcium and nitrates were shifted to the nutrient solution containing nitrates but lacking calcium. Hourly examination of these plants for nitrates, day and night for a period of five days after supplying nitrates, showed that during that period nitrates were not absorbed. Only after seven days was a minute trace of nitrates found in the roots, and later a little in the tops of the plants.

The fact that Dr. Eckerson found practically no reducase in the roots of these plants nor in the roots of any of the calcium-deficient plants would seem to preclude the possibility of absorption and simultaneous complete assimilation of nitrates. Further, no nitrates and practically no ammonia were found in these plants at any time. In connection with reducase activity attention may be called to the relatively low phosphorus content of the roots of the calcium-deficient plants (tables VI and VII).

Although these plants of the minus-calcium minus-nitrate series did not absorb nitrates, they absorbed calcium instantly when shifted to the complete nutrient treatment. In forty-five minutes the entire root system contained uncombined calcium, and half an hour later there was a considerable quantity of calcium throughout the tops. It was, however, twelve

hours after the shift to plus-calcium treatment before these plants began to absorb nitrates from the solution, and about sixteen hours before faint traces of nitrites were observed.

Darkness treatment

An attempt was made to study the hydrolysis and re-utilization of substances in darkness. On July 1, some of the plants that had been grown in the solution deficient in calcium only, were shifted from the greenhouse to a dark room that was maintained at about 23° C. The plants were kept in continuous darkness until July 9. During that period the stems elongated several inches, and the previously formed upper leaves turned from yellow to green, although newly formed leaves were yellow. As is indicated in tables IV and V there was an increase in concentration of nitrates. Further, microchemical tests showed that after the darkness treatment there was considerable uncombined calcium present, whereas other plants of the same series in the light contained practically no uncombined calcium.

It is apparent from tables II and III that carbohydrates decreased greatly during the period of darkness, yet there was much starch even in the tissues of the stem and leaves that were newly formed in darkness. The gross macrochemical analyses of stems (tables IV and V) show little change in percentage of protein or other elaborated nitrogen. There must have been, however, very definite changes in these substances, otherwise the rapid growth of meristem of the stem and the elongation of cells could not have occurred. Unfortunately macro-methods for proteins do not differentiate between those of the meristem and those of mature tissue. Microchemical tests showed, however, that during the period in darkness the granular masses of protein material (page 612) practically disappeared and much meristematic tissue of stems and roots developed. In these regions there were also observed appreciable quantities of asparagine.

On July 1, some of the plants that had been grown with no external supply of either calcium or nitrates were also placed in darkness. The same growth responses occurred. With the exception that nitrates were absent, similar chemical changes also took place, including the accumulation in the plants of considerable uncombined calcium that was not present in plants of the same series in the light.

After the darkness treatment, the plants (minus-calcium-minus-nitrate) were given a solution containing nitrates, but as before no calcium. Nitrates were observed in the small roots within fifteen minutes and throughout the plant within one hour. Such a rapid intake of nitrates was in striking contrast to the lack of absorption by plants of the same series in the light.

Plants deficient in nitrates

The fourth series of plants, those that were grown with no external supply of nitrates but with an abundance of calcium and other essential elements (plus-calcium-minus-nitrate solution, table I) were typical nitrogen-deficient plants (**13, 27, 40**). All the leaves, but especially the lower ones, were yellowish green with purple veins. The stems were hard and woody, very high in starch and free of nitrates.

These plants when shifted to minus-calcium-plus-nitrate treatment absorbed nitrates instantly. In less than an hour nitrates were present in all parts of the plant, and as usual (**13, 40, 41**) nitrites were observed in large quantities for three or four hours following the initial absorption of nitrates.

At the time of shift in nutrient treatments, the plants were observed for calcium distribution. Parenchymatous cells that were filled with masses of calcium oxalate crystals were conspicuous in phloem, cortex and pith. They were present in large numbers even in the extreme tip of the stem, and in parenchymatous cells of all parts of the root system. After transfer from the plus-calcium-minus-nitrate to the minus-calcium-plus-nitrate solution there was within a few days and for only a short time renewed vegetative growth. The concentration of uncombined calcium remained practically constant for about ten days, although there was a steady decrease in combined calcium during the same period. At the end of ten days, masses of calcium oxalate crystals were conspicuous in the base and middle of the stem, but none were present within five, and very few within ten, centimeters of the tip. The disappearance of calcium oxalate masses seemed to be first from the phloem and later from the cortex and pith. At the end of four weeks the concentration of combined calcium was very low, there was practically no uncombined calcium, and the stem tips were practically dead. Yet at that time calcium oxalate deposits were observed in the base of the stem and to a much lesser degree in the lower petioles. In many instances the masses looked as if they were undergoing decomposition.

Thus the re-utilization of calcium oxalate proceeded slowly and incompletely from the tip backward, and the plants died while crystals of calcium oxalate were still present in the base of the stem.

Discussion

If seedling tomato plants are transplanted to sand cultures deficient respectively in nitrogen, phosphorus, or potassium, the effects of nitrogen- or phosphorus-deficiency are evident in a comparatively short time (**13, 40, 15**), but conspicuous symptoms of lack of potassium (**41**) are not usually

apparent until much later. Although the symptoms of lack of these respective elements may not occur simultaneously, the effects upon the general appearance of tomato plants are similar. The lower leaves and lower stem are yellowish green tinged with the purplish blue of anthocyanin pigments, and the uppermost leaves and tip of the stem are fairly dark green and may remain so for a considerable period.

On the other hand, the appearance of the calcium-deficient plants of these experiments was very distinctive, unlike tomato plants deficient in nitrogen, phosphorus, or potassium, in that the upper part of the plant was yellow rather than green, and the lower half instead of being yellowish with practically dead leaves was fairly dark green.

Closely following the lack of chlorophyll in the tops, a condition which has been observed even in the lower plants (**5, 17, 45, 46**), there was a cessation of meristematic activity and death of tissue. This may, of course, occur when any essential element is deficient but it occurred in the tomato plants of these experiments within three weeks of minus-calcium treatment. Similar observations on tobacco plants were made by GARNER (**17**), who found that a lack of calcium affected most seriously the upper leaves and embryonic tissues of the growing point. He found that deficiency of magnesium, like deficiency of nitrogen, phosphorus or potassium, affected particularly the lower half of the plant.

The peppery brown spotting observed in the leaves of some of the calcium-deficient plants was probably iron toxicity, and was not due directly to insufficient calcium. Certainly it is not a distinguishing characteristic, although others (**17, 43**) have reported that these symptoms occurred when plants were grown in a deficiency of calcium. Analyses of leaf blades of the several series showed that there was often somewhat more filterable iron in the minus- than in the plus-calcium plants. Furthermore, the peppery spotting was easily obtained at will in plants of any of the series of these experiments but particularly in the calcium-deficient plants, simply by adding ferrous sulphate to the nutrient solution at the rate of ten or twenty milligrams of iron per liter. Respective tissues of calcium-deficient plants were not, however, more acid than those of the other series (page 608). If they had been, the higher percentage of soluble iron might be explained on the basis of SHIVE's (**53**) results, for he has found that soluble iron is relatively high when cell sap is acid, relatively low when cell sap is more alkaline. On the other hand experimental work by SMYTH (**54**) has indicated that quality of acid may be an important factor in its influence upon the form of iron present, and work by GARNER (**17**) suggests that mineral deficiencies may directly or indirectly affect the quality of organic acids in a plant. In our experiments specific acids were not determined.

In view of popular theories (**57**) it also appeared possible that magnesium toxicity might be a factor in the growth responses obtained. However, when concentrations of magnesium sulphate more than three times as high as that of the regular nutrient solutions (table I) were applied for a period of two weeks to some of the plants not employed for analysis or other purposes, no spotting of the leaves nor any other visible effect upon the plants was observed in any of the series. The original total molecular concentration of the nutrient solutions was maintained by decreasing the concentration of potassium phosphate in proportion to the increase in magnesium sulphate. Likewise a few plants of the several series were grown for two weeks with no external supply of magnesium by substituting potassium sulphate for magnesium sulphate in equivalent concentration in the nutrient solution. The temporary removal of magnesium from the nutrient solution produced no visible effect on the growth responses of the plants. It is also apparent from tables VI and VII that there was no marked excess nor deficiency of magnesium in the minus- as compared with the plus-calcium plants. Further, PFEIFFER (**42**) shows that in oat plants the ratio of calcium to magnesium may vary within rather wide limits with no noticeable effect upon the plant.

It has been reported (**10, 55**) that lack of abundant calcium results in short, stubby, bulbous roots that are characteristically brown at the tips, with sloughing off of cells further back. The calcium-deficient tomato plants of these experiments were no exception. Internally there was differentiation of xylem and phloem practically down to the embryonic tip, which means, of course, that they were growing very slowly as compared with the plus-calcium roots, in which active division and elongation of cells resulted in a long region of elongation between embryonic tip and region of maturation. In the stubby tips of the calcium-deficient plants, however, distortions and irregular planes of division mentioned by SOROKIN and SOMMER (**55**) were not evident. The cells seemed entirely regular, although particularly in the cortex the cells enlarged somewhat more laterally than longitudinally.

The conspicuous browning of roots, a symptom very commonly associated with calcium deficiency, was due mainly to browning of meristem and parenchymatous tissue of the cortex. The stele seemed little affected and there were numerous root hairs that did not appear materially different from those of the plus-calcium plants except that in general they were somewhat shorter. The browning was mostly of the cell walls, which in healthy parenchyma are considered to be composed mainly of cellulose and pectic materials. The nature of change that resulted in browning of the cell walls was not determined. It is not uncommon, however, in tomato roots and may be produced by many types of injury.

No definite information was secured as to the presence or absence of a middle lamella of calcium pectate. After treatment with oxalic acid, rows of small calcium oxalate crystals were frequently observed along cell walls in the cortical tissue of roots of the plus-calcium plants, possibly indicating the presence of a middle lamella of calcium pectate. Although such a reaction was not invariably obtained in all cells even in the plants grown with an abundance of calcium, yet on the other hand, it was not observed at all in roots of the calcium-deficient plants that showed much browning and sloughing of outer cortical cells. It has, however, been reported by others (**36, 45, 61**) that in a deficiency of calcium a middle lamella of calcium pectate may not develop.

Whereas the browning of roots was mainly in cell walls, the protoplasts of the meristem and older tissue also became decidedly granular, and this mass was usually golden brown in color and gave strong protein reactions. Apparently in a deficiency of calcium, as in a lack of phosphorus (**15**) or potassium (**41**), there is definite interference with formation of proteins essential to the protoplasts of active cells.

Many different workers (**4, 19, 22, 52**), using various kinds of plants have found that carbohydrates frequently accumulate in the tissues of plants deficient in calcium. It has been shown, however, (page 617) that calcium limitation directly or indirectly interferes with chlorophyll formation. Therefore, under certain experimental conditions and in a deficiency of calcium the formation of chlorophyll might be so limited as to seriously decrease the rate of assimilation of carbon dioxide and thereby prevent accumulation of carbohydrates. This was possibly the case in results reported by BURRELL (**6**). Nevertheless, calcium deficiency seems quite commonly to result in carbohydrate accumulation, and the enormous concentration of sugars and starch in the low calcium plants of these experiments is clearly shown in tables II and III. Microchemical reactions also showed that starch was present in great abundance throughout all parenchymatous tissues, even in the tips of roots and stems.

In explanation of such observations it is usually said that calcium is essential for digestion of starch and translocation of sugars, and that in a lack of this element carbohydrates therefore accumulate. But it is evident that there is no sound basis for this notion, because in the calcium-deficient plants of these experiments sugar and even starch grains were found in the most distal portions of roots, stems and leaves. In what manner translocation of carbohydrates might be more complete is indeed a question. Also SCHIMPER (**52**) has shown that digestion of starch and translocation of sugars take place in leaves and stems of plants that contain a very low concentration of calcium. Further, the minus-calcium tomato plants of

these experiments after a few days in darkness decreased greatly in carbohydrates (tables II and III). Likewise, microchemical reactions showed the presence of an abundance of sugar and starch in tissues of stem and leaves that were yellow in color and newly formed in darkness. Thus there is additional evidence showing that there may be digestion of starch, translocation of sugars and re-condensation of sugars to starch in plants extremely low in calcium (tables II and III). It should be pointed out, however, that during the period in darkness there was an increase in uncombined calcium (page 615), although it does not appear probable that this was a direct factor in effecting digestion of starch and translocation of sugars, for the latter process began to occur considerably in advance of any apparent change in form of calcium.

One of the principal uses of carbohydrates is in protein synthesis. It has been shown repeatedly (**13, 27, 39, 40**) that carbohydrates may accumulate if a plant has no external supply of available nitrogenous nutrient. They may even accumulate with nitrates present in abundance in the nutrient medium or even in the tissue of the plant, if there is little reducase activity due to a deficiency of potassium (**41**) or phosphorus (**15, 28**). Both elements are necessary for synthesis of nitrates to protein.

When the plants were shifted from soil to sand culture at the beginning of these experiments all were in effect plus-calcium plants. At that time and for a short period thereafter, there was presumably some protein synthesis by the plants that were receiving minus-calcium nutrient treatment. However, carbohydrates undoubtedly accumulated in these plants because the amount of protein synthesis was small.

There are two reasons for the small amount of protein synthesis. First, the plants were very much limited in their ability to absorb nitrates (page 611), and second, the small quantity of nitrates absorbed (tables IV and V) remained for the most part unelaborated, as Dr. ECKERSON found that the calcium-deficient plants, especially the roots, were very low in reducase (nitrate reducing material). Apparently there was little synthesis of even the simpler organic compounds of nitrogen. The minus-calcium plants increased very little in volume. Accordingly, if there had been synthesis of simpler forms of organic nitrogen or protein it would be evident on a percentage-of-green-weight basis; but table V indicates that this did not occur. The effects of phosphorus (**15, 28**) or potassium (**41**) deficiency are somewhat different.

The concentration and quality of the various nitrogenous fractions of the whole stems were about the same in plus- as in minus-calcium plants (tables IV and V), yet microchemical reactions and anatomical examination showed decided differences. There was comparatively little meristematic

tissue in roots or stems of the calcium-deficient plants, but there were present in the region of phloem and inner cortex of roots and stems, cells which contained golden brown granular masses of material that was in large part proteinaceous. These protein-like inclusions may not be peculiar to calcium deficiency, as a somewhat similar condition may occur in a lack of phosphorus (**15**) or potassium (**41**), but apparently the very early death of embryonic tissue is a factor invariably accompanying a lack of calcium (**10, 17, 52, 55**). This element appears to be directly or indirectly necessary for development of proteins essential to the protoplasts of active cells. Without calcium, proteinaceous inclusions accumulate, not only in tomato but in other plants (**58**).

It is not apparent why a deficiency of calcium almost completely prevented absorption of nitrates (page 614), when the same plants that failed to absorb nitrates took in calcium instantly. The experimental results fail to indicate whether or not calcium deficiency limits the absorption of materials other than nitrates. Attention may, however, be called to tables VI and VII. The concentration of sulphur is much the same in both plus- and minus-calcium plants. Both lots of plants also contain about the same percentage of magnesium with the exception of the petioles of the calcium-deficient plants, which are comparatively low in this element. The concentration of potassium is comparatively high in the plants lacking calcium. This is a condition which has often been observed by others (**31, 32, 47**). It should be remembered, however, that the minus-calcium nutrient solution (table I) was comparatively high in potassium.

There seems to be nothing in the literature which has any very significant bearing on the inability of the minus-calcium plants to absorb nitrates,[2] although it is well known (**18, 29, 30**) that leguminous plants especially are high in nitrogen if the soil is abundantly supplied with calcium. The root systems of the low-calcium tomato plants were, of course, abnormal (pages 610 and 614) yet other essential elements were absorbed. The usual theories (**57**) on permeability are, in fact, opposed to the results obtained. Also, work by TRUE (**61**) and ECKERSON has shown that certain materials may be absorbed more readily by plants

[2] It seems impossible to draw conclusions from the work of JERMAKOW (23). He placed excised leaves in plus- and minus-calcium solutions and analyzed the leaf tissue for nitrates. At least as far as absorption is concerned, excised leaves can scarcely be compared with an intact root system. He found, however, a greater concentration of nitrates in the tissues of the minus-calcium leaves. He assumes, therefore, that there was more rapid assimilation (disappearance) of nitrates in the leaves which received an external supply of calcium. He did not, however, analyze his residual nutrient solutions, nor did he determine other nitrogenous fractions. He has therefore no measure of nitrates absorbed. Further, his leaves were presumably obtained from soil-grown plants, and it is doubtful if they were deficient in calcium.

deficient in calcium than by others in a full nutrient solution. These workers did not, however, determine the ability of a calcium-deficient plant to absorb nitrates.

Whether or not the low phosphorus content of the roots of the minus-calcium plants was a factor affecting nitrate absorption is not apparent. Only total phosphorus was determined.[3] Although phosphatides are said to be important in determining the permeability of wall or protoplast (**57**) the question is at best unsettled (**56**), and little can be gained by discussion. It may, however, be pointed out that CHIBNALL (**8**) reports the presence of a calcium phosphatidate in cabbage.

In connection with the low content of total phosphorus in the calcium-deficient roots, it may be emphasized that there rather than in the tops reducase was lowest. Also, ECKERSON'S work (**15**) indicates that a lack of phosphorus limits reducase activity apparently more than a deficiency of other essential elements.

A series of excellent experiments by THERON (**59**) showed that the removal of nitrates was less from an alkaline than from an acid solution. Likewise SABININ and KOLOTORA (**51**) obtained somewhat similar results. However, in recent work (**50, 60**) with the tomato the pH of the nutrient medium did not materially influence absorption until the plant became apparently saturated with nitrates. In an alkaline medium, nitrate saturation within the plant occurred in a very short time; thereafter the plants did not absorb nitrates. The plants grown at a high pH had the ability to *absorb* nitrates but were unable to synthesize them to amino acids or protein.

The literature (**9, 11, 37, 59, 62**) is full of contradictory statements as to the effect of calcium deficiency on the pH of plants. Where whole tops are employed as a sample, the pH is usually lower in the calcium-deficient plants than in the more vigorously growing plants that have had an adequate supply of calcium; the difference can be accounted for by the fact that there is a larger amount of meristematic tissue, notably alkaline (**20**), in the latter. However, in homologous fractions of plus- and minus-calcium tomato plants the tissue and expressed sap were not materially different. The pH of nutrient solutions was also essentially the same in each of the respective series, practically optimum for maximum absorption and assimilation of nitrates (**50, 60**). Accordingly it does not seem probable that pH of plant or of nutrient solution was a limiting factor in the inability of the minus-calcium plants to absorb nitrates.

The failure of the calcium-deficient plants to absorb nitrates does not appear possible of explanation at this time. It is significant, however, that there was very rapid absorption of nitrates in low-calcium plants that had been subjected to a period of darkness (page 615). Within the minus-

[3] Microchemical tests for calcium phosphate gave negative results.

calcium plants conspicuous changes were associated with darkness treatment; these included proteolysis involving the loss of many of the granular proteinaceous inclusions, decrease in combined calcium, and increase in uncombined calcium. RISSMAN (**49**) also records a decrease in concentration of insoluble calcium for wheat grown in darkness, and RAMANN'S (**44**) results indicate that there is more mobile calcium at night than during the day. The chemical significance of this change in form of calcium is not known. It is obvious that uncombined calcium alone (at least when newly absorbed) was not sufficient to result directly and immediately in nitrate absorption, for much uncombined calcium was present throughout the minus-calcium plants one hour after the shift to plus-calcium treatment. Nitrates, however, as determined by their complete absence and by lack of reduction products, were not absorbed until twelve hours later.

Apparently before nitrate absorption could take place other changes were necessary, changes probably involving the combination of calcium with protein or other materials. It is doubtful if proteolytic changes were a direct factor in either case, as only twelve hours of plus-calcium treatment were required before nitrates were absorbed. During such a short period of time there could scarcely have been extensive synthetic or proteolytic changes. But proteolysis or other catabolic changes in darkness released combined calcium. In another part of the experiment uncombined calcium was furnished by an external supply of soluble calcium salts. In both cases there apparently followed a re-combination with newly formed proteins or other materials. A supply of amino acids for formation of new protein was available in both instances; in the minus-calcium plants in darkness, through protein decomposition to amino acids, and in the plants newly supplied with calcium, through nitrate reduction to amino acids (page 615).

Non-absorption of nitrates was not a direct effect of a poorly proportioned nutrient solution, as the series of plants containing calcium but lacking nitrates (plus-calcium minus-nitrate, table I) absorbed nitrates instantly from the minus-calcium plus-nitrate solution.

Neither was darkness a direct factor in effecting nitrate absorption by the calcium-deficient plants. Moderate shading produced the same results but in lesser degree. Undoubtedly cloudy weather would tend to have the same effect, and probably the short days of winter in case of tomato (**38, 41**). All these conditions are apparently essentially similar in that they result in proteolysis, in a decrease in carbohydrates, and in the release of combined calcium for recombination with material of the protoplast. Likewise, if the initial plants of these experiments had been very low in carbohydrates and high in the simpler forms of organic nitrogen (**40**), a greater degree of re-utilization, or less combination of calcium might have taken

place and different results might have been obtained with respect to nitrate absorption. This point is to be investigated. However, a low concentration of organic nitrogen accompanied with a high percentage of carbohydrates in the plant does not in itself inhibit nitrate absorption. The plants of these experiments that were high in calcium and deficient in nitrates were low in all forms of nitrogen and extremely high in carbohydrates, yet they absorbed nitrate in abundance. But they also contained an abundance of uncombined calcium, which was available for combination with materials of newly formed cells. Also they were very high in reducase activity, as is usual for plants of such quality.

There appears to be but fragmentary evidence as to the forms of calcium found in plants, and much of this is misleading on account of the fact that air dried tissue has been employed for analysis (**2, 25, 49**). KOSTYTSCHEW and BERG (**25**) report that calcium does not occur in organic combination with protein. Their results, however, do not warrant this conclusion.[4] On the other hand, there is apparently no proof that calcium combines with proteins, although in the body fluids of animals it is said (**35**) to form a non-ionized protein compound. It would, however, appear significant that increase in uncombined calcium closely paralleled changes in the minus-calcium tomato plants which must have involved proteolysis (page 615). There may, of course, have been significant changes in other materials such as possibly phosphatidates of calcium. There is, however, increasing evidence tending to indicate that mineral elements may exist in combination with protein (**24, 33, 35, 54, 63**).

There are available many determinations (**21, 48**) of total water-soluble calcium. Such results, however, tell nothing as to the actual calcium compounds present. In many cases calcium oxalate undoubtedly accounts for much of the insoluble calcium fraction, and probably calcium phosphate under some conditions. Also much of the insoluble calcium is present as *combined calcium* (page 607). The proportion of soluble and insoluble calcium varies (**21, 48**), yet there seems always to be considerable insoluble calcium, whereas potassium is practically all soluble and readily translocated from mature to embryonic regions (**41**).

In calcium-deficient plants, however, it has been observed that most of the calcium present is in the older tissue of roots and tops (**17, 21, 46, 61**). Likewise, the low-calcium tomato plants (tables VI and VII) contained con-

[4] KOSTYTSCHEW and BERG first dried plant tissue, apparently at room temperature, and extracted with cold water. Yet it has been shown by CHIBNALL (7) and others that such treatment results in extensive proteolytic changes; changes which in the tomato at least are associated with liberation of combined calcium. Following aqueous extraction these workers treated the tissue with 2N acetic acid and 2N hydrochloric acid. As might be anticipated, the ash of the residue was calcium-free, as either of the reagents employed react with the usually recognized calcium constituents of the plant.

siderable calcium in the lower half of the stem, yet there was only a trace in the upper stem. In this connection may be recalled the external appearance of the calcium-deficient plants, yellow in the upper half of the tops but green below. In contrast, in plants that are deficient in phosphorus (**15**), nitrogen (**14, 27, 40**), or potassium (**41**), the lower leaves are yellowish but the tips are comparatively green, and the greatest concentration of these elements is found in the tips or in other embryonic tissue (**15, 27, 40, 41**). Further, practically all the calcium present in fresh tissue of the minus-calcium tomato plants was found to be water-insoluble. Some of it was present as calcium oxalate deposits in mature tissues, but much of the insoluble calcium was in another form (combined calcium) that reacted with oxalic acid only after treating sections with alkali, or after the plants had been subjected for several days to continuous darkness or shading.

It has been shown by various workers (**26, 34**) that there may be to some extent re-utilization of calcium oxalate. The low-calcium tomato plants utilized calcium oxalate (page 616), but the crystals were dissolved by the plant so slowly that the embryonic tissue of stem and root tip died while there were yet heavy deposits of calcium oxalate in the base of the stem and in the lower petioles.

In addition to calcium oxalate crystals, there was combined calcium in older tissue of low-calcium plants when they died. Stimultaneously, however, with disappearance of uncombined calcium there was death of meristematic tissue. When new cells are formed there must be not only calcium presumably for the middle lamella, but available calcium for combination with materials of the protoplast. It seems quite probable that some of this material may be protein, as without calcium inclusions accumulate in the protoplast in the form of granular masses that are, at least in large part, constituted of proteins (page 612).

Summary

1. The upper parts of the tops of calcium-deficient tomato plants were yellow and the lower leaves and stems were fairly green. (In a deficiency of nitrogen, phosphorus or potassium respectively, the lower portion of the plant is yellowish, but the upper leaves and stem remain green.)

2. The roots were characteristically short, bulbous and brown at the tips, with sloughing off of cells further back. The roots were short because of slow growth of the meristem, and bulbous because cortical cells enlarged somewhat more laterally than longitudinally. The failure of lateral root primordia to develop also contributed to the bulbous appearance.

3. Sloughing off of cells was probably due in part to the fact that a middle lamella of calcium pectate did not develop in roots of tomato plants extremely deficient in calcium.

4. Browning of the roots occurred in the cell wall and in the protoplast. The latter was composed in part of granular proteinaceous material. Such protoplasts were also present in the stem, especially near the meristem.

5. Calcium-deficient tomato plants of these experiments under the seasonal light conditions of the greenhouse were practically unable to absorb or assimilate nitrates although they absorbed calcium instantly.

6. The plants which lacked calcium accumulated carbohydrates in large quantities, apparently because absorption and assimilation of nitrates did not take place.

7. Translocation of sugars and digestion of starch took place freely in tomato plants extremely low in calcium.

8. Nearly 100 per cent. of the calcium of fresh tissue of the calcium-deficient plants was water-insoluble and most of it was located in older tissues of roots and tops. (In a deficiency of nitrogen, phosphorus, or potassium respectively, the highest concentration of the deficient elements is in young embryonic tissues.)

9. Some of this insoluble calcium was present as calcium oxalate, but much was in another form, *combined calcium,* that reacted with oxalic acid only after treatment with alkali.

10. In calcium-deficient tomato plants utilization of calcium oxalate and re-utilization of *combined calcium* took place, but so slowly that root and stem tips died while there were yet heavy deposits of calcium oxalate and a high concentration of *combined calcium* in older tissues.

11. When new cells are formed there must be calcium not only presumably for the middle lamella, but also available calcium for combination with materials of the protoplast. Otherwise granular proteinaceous inclusions accumulate.

12. Calcium-deficient tomato plants that were shaded or placed in continuous darkness for several days decreased in carbohydrates. Associated with the decrease in carbohydrates there was proteolysis and a noticeable increase in *uncombined calcium* and a diminished concentration of *combined calcium.*

13. Accompanying proteolysis and increase in *uncombined calcium* there was rapid formation of new stem tissue and absorption of nitrates, even though there was no external supply of calcium available.

14. Calcium-deficient plants in the light were given an external supply of soluble calcium salts. A few hours after absorption of calcium there was absorption and assimilation of nitrates, and subsequently growth was resumed.

15. Darkness treatment of calcium-deficient plants, and shift from minus- to plus-calcium nutrient, are apparently in certain respects similar in principle and effect. In both cases there is made available *uncombined calcium* for combination with newly formed proteins or other materials. A supply of amino acids for formation of new proteins of meristems is like-

wise available in both instances: in the calcium-deficient plants in darkness, through proteolysis, and in the plants newly supplied with calcium, through nitrate assimilation.

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LITERATURE CITED

1. ALLEN, C. E. On the origin and nature of the middle lamella. Bot. Gaz. **32**: 1–34. 1901.
2. ASO, K. On the different forms of lime in plants. Bull. Coll. Agr. Tokyo Univ. **5**: 239–242. 1902.
3. Association of Official Agricultural Chemists. Official and tentative methods of analyses. 2nd ed. Washington, D. C. 1925.
4. BOEHM, J. Ueber den vegetabilischen Nährwerth der Kalksalze. Sitzber. Wien. Acad. Wiss. **71**: 287–304. 1875.
5. BOKORNY, T. Ueber den Einfluss des Calciums und Magnesiums auf die Ausbildung der Zellorgane. Bot. Centralbl. **62**: 1–4. 1895.
6. BURRELL, R. C. Effect of certain deficiencies on nitrogen metabolism of plants. Bot. Gaz. **82**: 320–328. 1926.
7. CHIBNALL, A. C. Investigations on the nitrogenous metabolism of the higher plants. III. The effect of low temperature drying on the distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 599–610. 1922.
8. ————, and CHANNON, J. H. The ether-soluble substances of cabbage leaf cytoplasm. VI. Summary and general conclusions. Biochem. Jour. **23**: 176–184. 1929.
9. CLEVENGER, C. B. Hydrogen-ion concentration of plant juices. II. Factors affecting the acidity or hydrogen-ion concentration of plant juices. Soil Sci. **8**: 227–242. 1919.
10. DAY, D. Some effects of calcium deficiency on *Pisum sativum*. Plant Physiol. **4**: 493–506. 1929.
11. DUSTMAN, R. B. Inherent factors related to absorption of mineral elements by plants. Bot. Gaz. **79**: 233–264. 1925.
12. ECKERSON, S. H. Microchemical studies in the progressive development of the wheat plant. Washington Agr. Exp. Sta. Bull. 139. 1917.
13. ————. Protein synthesis by plants. I. Nitrate reduction. Bot. Gaz. **77**: 377–390. 1924.
14. ————. Properties of the nitrate-reducing substance in plants. Contributions from the Boyce Thompson Inst. (Unpublished.) Abst. Amer. Jour. Bot. **16**: 851–852. 1929.
15. ————. Influence of phosphorus deficiency on metabolism of the tomato. Contributions from the Boyce Thompson Inst. **3**: 197–217. 1931.

16. ————————. Microchemistry (mimeographed).
17. GARNER, W. W., MCMURTREY, J. E., BOWLING, J. D., and MOSS, E. G. Magnesium and calcium requirements of the tobacco crop. Jour. Agr. Res. **40**: 145–168. 1930.
18. GINSBURG, J. M., and SHIVE, J. W. The influence of calcium and nitrogen on the protein content of the soybean plant. Soil Sci. **22**: 175–197. 1926.
19. GRAFE, V., and VON PORTHEIM, L. Untersuchungen über die Rolle des Kalkes in der Pflanze. Anz. k. k. Akad. Wiss. Wien. Math-nat. Kl. **43**: 324–325. 1906. (See also Sitzber. Akad. Wiss. Wien. Math-Nat. Kl. **115**: Abt. I. 1003–1037. 1906.)
20. HAAS, A. R. C. Studies on the reaction of plant juices. Soil Sci. **9**: 341–368. 1920.
21. ————————. Water-solubility of dry matter in relation to calcium nutrition of normal orange and lemon leaves. Bot. Gaz. **85**: 334–340. 1928.
22. HARTWELL, B. L. Starch congestion accompanying certain factors which retard plant growth. Rhode Island Agr. Exp. Sta. Bull. 165. 1916.
23. JERMAKOW, W. W. Zur Frage über die Beziehungen zwischen den Salzen des Calciums und der Assimilation des Nitratstickstoffs. Russ. Jour. Expt. Landw. **6**: 403–431. 1905.
24. KLEIN, G. Der Mikrochemische Nachweis von organisch gebundenem Schwefel und Magnesium in der Pflanze. Oesterr. Bot. Ztschr. **76**: 15–24. 1927.
25. KOSTYTSCHEW, S., and BERG, V. Die Form der Calciumverbindungen in lebenden Pflanzengeweben. Planta, **8**: 55–67. 1929.
26. KRAUS, G. Ueber das Verhalten der Kalkoxalats beim Wachsen der Organe. Flora **83**: 54–73. 1897.
27. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
28. KRAYBILL, H. R. Unpublished work dealing with some relationships of phosphorus to plant growth and metabolism.
29. LIPMAN, J. G., and BLAIR, A. W. The yield and nitrogen content of soybeans as influenced by lime. Soil Sci. **4**: 71–77. 1917.
30. ————————, and ————————. Field experiments on the availability of nitrogenous fertilizers, 1908–1917. Soil Sci. **9**: 371–392. 1920.
31. ————————, ————————, and PRINCE, A. L. The effect of lime and fertilizers on the potash content of soil and crop. International Rev. Sci. and Practise of Agr. **4**: 546–553. 1926.

32. LOEHWING, W. F. Calcium, potassium and iron balance in certain crop plants in relation to their metabolism. Plant Physiol. 3: 261–275. 1928.
33. LUTMAN, B. F., and WALBRIDGE, N. L. The rôle of magnesium in the aging of plants. Vermont Agr. Exp. Sta. Bull. 296. 1929.
34. MAJUMDAR, G. P. Some observations on the distribution of calcium oxalate crystals in plant tissues, and their probable rôle in plant metabolism. Indian Sci. Cong. Proc. (Calcutta) **12**: 187–188. 1925.
35. MARRACK, J., and THACKER, G. The state of calcium in body fluids. Biochem. Jour. **20**: 580–594. 1926.
36. MOLISCH, H. Mikrochemie der Pflanzen. 3rd ed. Jena. 1923.
37. NEWTON, J. D. A comparison of the absorption of inorganic elements, and of the buffer systems of legumes and non-legumes, and its bearing upon existing theories. Soil Sci. **15**: 181–204. 1923.
38. NIGHTINGALE, G. T. The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. 74. 1927.
39. ————, and ROBBINS, W. R. Some phases of nitrogen metabolism in polyanthus narcissus (*Narcissus tazetta*). New Jersey Agr. Exp. Sta. Bull. 472. 1928.
40. ————, SCHERMERHORN, L. G., and ROBBINS, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.
41. ————, ———— and ————. Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. New Jersey Agr. Exp. Sta. Bull. 499. 1930.
42. PFEIFFER, T., and RIPPEL, A. Der Einfluss von Kalk und Magnesia auf das Wachstum der Pflanzen. Jour. Landw. **68**: 5–39. 1920.
43. VON PORTHEIM, L. R., and SAMĚC, M. Über die Verbreitung der unentbehrlichen anorganischen Nährstoffe in den Keimlingen von *Phaseolus vulgaris*. Flora **94**: 263–286. 1905.
44. RAMANN, E. Mineralstoffgehalt von Baumblättern zur Tages- und zur Nachtzeit. Jahrb. wiss. Bot. **50**: 84–91. 1911.
45. REED, H. S. The value of certain nutritive elements to the plant cell. Ann. Bot. **21**: 501–543. 1907.
46. ————, and HAAS, A. R. C. Effect of sodium chloride and calcium chloride upon growth and composition of young orange trees. Univ. California Tech. Paper No. 4. 1923.
47. ————, and ————. Nutrient and toxic effects of certain ions on citrus and walnut trees with especial reference to the

concentration and pH of the medium. Univ. California Tech. Paper No. 17. 1924.

48. ————, and ————. Some relations between the growth and composition of young orange trees and the concentration of the nutrient solution employed. Jour. Agr. Res. **28**: 277–284. 1924.
49. Rissmann, R. Der Mineralstoffwechsel grüner and etiolierter Pflanzen unter besonderer Berücksichtigung des Magnesiums und der Chlorophyllbildung. Planta **9**: 195–245. 1929.
50. Robbins, W. R. Unpublished work dealing with the pH of the nutrient medium in relation to absorption and assimilation of nitrates.
51. Sabinin, D. A., and Kolotora, G. S. The character of the passage of ash substances into the plant. I. The reaction of the medium as a factor in the mineral nutrition of plants. Proc. Intern. Soc. Soil Sci. **4**: 81–82. 1929.
52. Schimper, A. F. W. Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73**: 207–261. 1890.
53. Shive, J. W. Plant physiology. New Jersey Agr. Exp. Sta. Ann. Rept. 1927. 30–32. 1927.
54. Smyth, C. V., and Schmidt, C. L. A. Studies on the mode of combination of iron with certain proteins, amino acids, and related compounds. Jour. Biol. Chem. **88**: 241–269. 1930.
55. Sorokin, H., and Sommer, A. L. Changes in the cells and tissues of root tips induced by the absence of calcium. Amer. Jour. Bot. **16**: 23–39. 1929.
56. Steward, F. C. On the evidence for phosphatides in the external surface of the plant protoplast. Biochem. Jour. **22**: 268–275. 1928.
57. Stiles, W. Permeability. New Phytologist. Reprint No. 13 (London). Wheldon and Wesley. 1924.
58. Stock, G. Ein Beitrag zur Kenntniss der Proteinkrystalle. Beitr. Biol. Pflanz. **6**: 213–235. 1892.
59. Theron, J. J. Influence of reaction on inter-relations between the plant and its culture medium. Univ. California Pub. Agr. Sci. **4**: 413–444. 1924.
60. Tiedjens, V. A. Unpublished work dealing with the pH of the nutrient medium in relation to absorption and assimilation of ammonium and nitrate salts.
61. True, R. H. The significance of calcium for higher green plants. Science **55**: 1–6. 1922.
62. Truog, E., and Meacham, M. R. Soil acidity. II. Its relation to the acidity of the plant juice. Soil Sci. **7**: 469–474. 1919.
63. Vickery, H. B., and Osborne, T. B. A review of hypotheses of the structure of proteins. Physiol. Rev. **8**: 393–446. 1928.

ELECTRIC CORRELATION BETWEEN LIVING CELLS IN CORTEX AND WOOD IN THE DOUGLAS FIR

E. J. LUND

(WITH SEVEN FIGURES)

In presenting the results of the experiments which follow in this paper the writer will, for the sake of simplicity and proper evaluation of the observations, assume that the reader is familiar with the facts of electric polarity in the Douglas fir which have been presented in previous papers (LUND **1, 2, 3**).

It will be recalled that when electrode contacts are made at the center of the wood and any point on the outer surface in the same cross-section of the tree, the center of the wood is always electropositive (in the external circuit) to the outer surface of the cortex. This is true for all regions of the tree stem below that of the first or second apical internode. Recent observations reported below indicate that the orientation of the radial polarity in the region of the most apical internode is reversed under conditions of absence of injury or stimulation.

Since all or at least the greater part of the radial P. D. is confined to the living parts of the wood-cortex system and since the cambium exhibits a bipolar structure which corresponds to the bipolar radial growth in wood and cortex, we might expect to find that the wood-cortex system including the cambium also exhibits a double electric polarity.

Up to the time of the present experiments and those to be presented in following papers on the effect of temperature, it had not been possible to demonstrate with certainty whether or not the cortex possessed an electric polarity of its own. The difficulty lay in the fact that any process of separation of the cortex from the wood axis involves more or less permanent injury, due to deformation of the cortex and therefore radical change in its electric polarity. This fact is also to be expected from the experiments reported in the preceding paper which pertain to the effect of mechanical stimulation on the electric polarity.

Various incidental observations seem to indicate that the radial E.M.F. *per se* in the wood is not affected to a noticeable degree by merely removing the cortex. This result might perhaps also be anticipated since no marked deformation of the wood occurs in such a process.

The preceding facts led the writer to devise a procedure by which the existence of the E.M.F. in the cortex could be made evident without injury or at least with a minimum of injury to the cortex.

The *principle of summation* of electric polarities of cells, which has been fully stated in previous papers, is obviously of fundamental impor-

tance. A proof of its validity and a demonstration of its rôle in electric correlation in the Douglas fir is one of the requirements, without which the writer's conception of the rôle of electric polarity of the cell in the process of correlation in tissues can not be considered established. The published evidence which appears to be conclusive is found in the experiments by MARSH (**7, 8**) on the onion root.

In the writer's first paper on the Douglas fir (LUND (**1**), pp. 10–11) and before we possessed any knowledge of the internal distribution of its correlation currents, it was assumed that the apparent algebraic summation of the external longitudinal E.M.F.'s along the stem was evidence for summation. This conclusion is of course correct provided it can be demonstrated that the origin of most or all of the observed external P.D. is not located at the surface of contact between the water leads of the electrodes and the external surface of the cortex. The experimental requirement was fully met in the experiments by MARSH on the onion root. In the following experiments it will now similarly be shown that the *removal of the cortex between the contacts without involving any disturbance of the contacts* themselves does produce characteristic and profound modifications of the E.M.F. similar to the results of the experiments by MARSH on the onion root. From these peculiar effects of the cortex on the E.M.F. we shall attempt to deduce in further detail the pattern of the correlation currents in the tree.

Experiments

The experiments reported here were carried out on the second and third internodes of the main axis of the tree. Each internode was isolated just before the beginning of the experiment. The lengths of the pieces varied from 50 to 70 cm. and the average diameters varied from 7 to 12 mm. The ratio of the length to the diameter was therefore actually about twice as great as that indicated in the diagrams of figure 1. Similarly in figure 6, the ratio of diameter to length of the internode is very much exaggerated for the purpose of illustration.

PROCEDURE I

Figure 1.I, curves, figure 2

In this experiment the third internode from a tree fifteen feet high was isolated and clamped rigidly at each end to a heavy iron stand. Dry cotton pads were wrapped around the jaws of the clamps to prevent mechanical injury to the tissues.

At points 10 cm. from each one of the cut ends of the internode, a ring of cortex 4 cm. wide, was removed. Holes two mm. in diameter were drilled to the center of the wood axis, at points midway between the cut

surfaces of the cortex. Into these holes were inserted glass funnels X and Z. The inner openings of the funnels were placed exactly in the center of the wood axis. These funnels were filled with tap water and served as electrode contacts at the center of the wood axis. Cotton strips saturated with tap water were wrapped around the wood axis and dipped into the cups A′, A and B, B′. These cups were filled with tap water and two movable Pb-$PbCl_2$ electrodes were hung over the edges of any pair of cups between which a measurement of P.D. was to be made.

In all the work care was taken to prevent excess wetting of the exposed wood axis by the cotton contacts. These contacts and the cups X and Z were rinsed occasionally to maintain symmetry. Flowing contacts gave the same results as contacts which were rinsed occasionally.

In the experiment illustrated by figure 1.I, the P.D. was measured between each pair of the contacts, X and A′, X and A, Z and B, Z and B′, X and B, Z and A, A′ and B′, A and B.

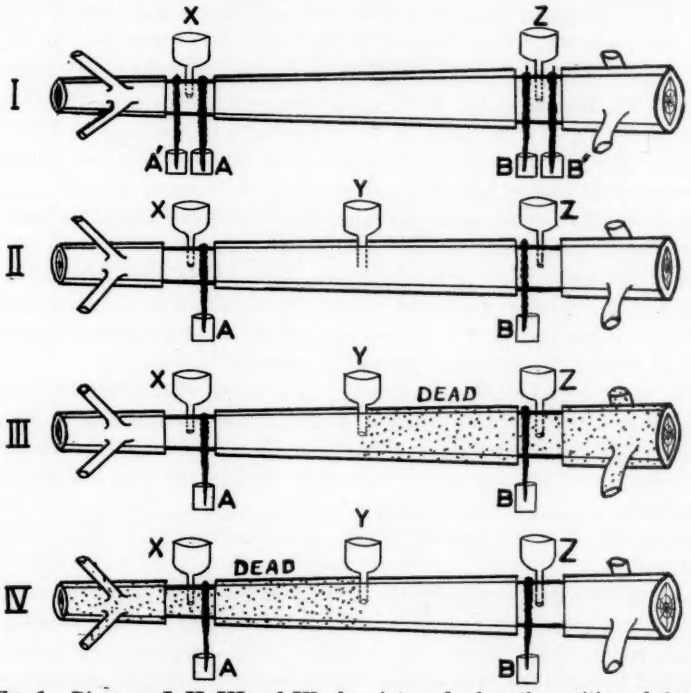

FIG. 1. Diagrams I, II, III and IV of an internode show the position of the glass funnel contacts X, Y and Z and the cotton contacts A and B in procedures of experiments I, II, III, IV respectively, which are referred to in the text. The stippled part of the stem was killed in boiling water before the tests were made.

The duration of one set of measurements by means of the potentiometer was about four to five minutes. The readings were always taken in the same sequence. Successive sets of readings were taken at variable intervals as indicated by the points on the curves in figure 2.

The following convention for designating the orientation of the electric polarity between the contacts will be followed throughout this and the following papers. The designations XB, AB, etc., on the curves mean respectively that contact X is electropositive in the external circuit to contact B; contact A is electropositive in the external circuit to contact B, etc., whenever the reading of the P.D. is above zero on the ordinate. Whenever the readings of P.D. fall below zero the contact designated by the first letter is electronegative to the contact designated by the second letter.

It will be observed in figure 1, and in the curves of figure 2 that:

(1) XA′, XA, and ZB, ZB′ measure radial E.M.F.'s in the wood.
(2) XB measures what will be called the apical-basal diagonal E.M.F.
(3) ZA measures what will be called the basal-apical diagonal E.M.F.
(4) AB and A′B′ measure what we shall call the longitudinal E.M.F.

The first fact to notice is that the sequence in the magnitude of the radial E.M.F.'s of the wood at the four pairs of contacts is ZB′ > ZB > XA > XA′. This relation is maintained during the whole period of the experiment and is merely a confirmation of previous measurements on the radial E.M.F. in which it was found that the radial E.M.F. of the wood diminishes as we proceed toward the apex of the tree.

In accordance with these facts we have arbitrarily designated the E.M.F. in the wood at the basal end of the internode in diagram figure 6 by 8 (±) signs and that in the apical end by 6 (±) signs. The significance of these arbitrary numbers will be referred to later.

The main object of the experiments is to show what effect the removal of the cortex between the contacts A and B has on the E.M.F. between each one of the different pairs of contacts. With this object in view three successive sets of measurements were made during the first thirty minutes of the experiment previous to removal of the cortex. During such a preliminary period there usually occurs a marked relative stabilization of the E.M.F.'s.

At the time designated by the vertical arrows the cortex between the contacts was gently but quickly stripped from the wood. During this process which lasted about two minutes the electrode contacts were not disturbed because the ends of the stem were fixed in rigid clamps. After the cortex was removed a reading was taken of each pair of contacts as designated in the curves, and thereafter the readings were taken at irregular intervals as indicated by the points on the curves.

INTERPRETATION OF THE CURVES

Figure 2

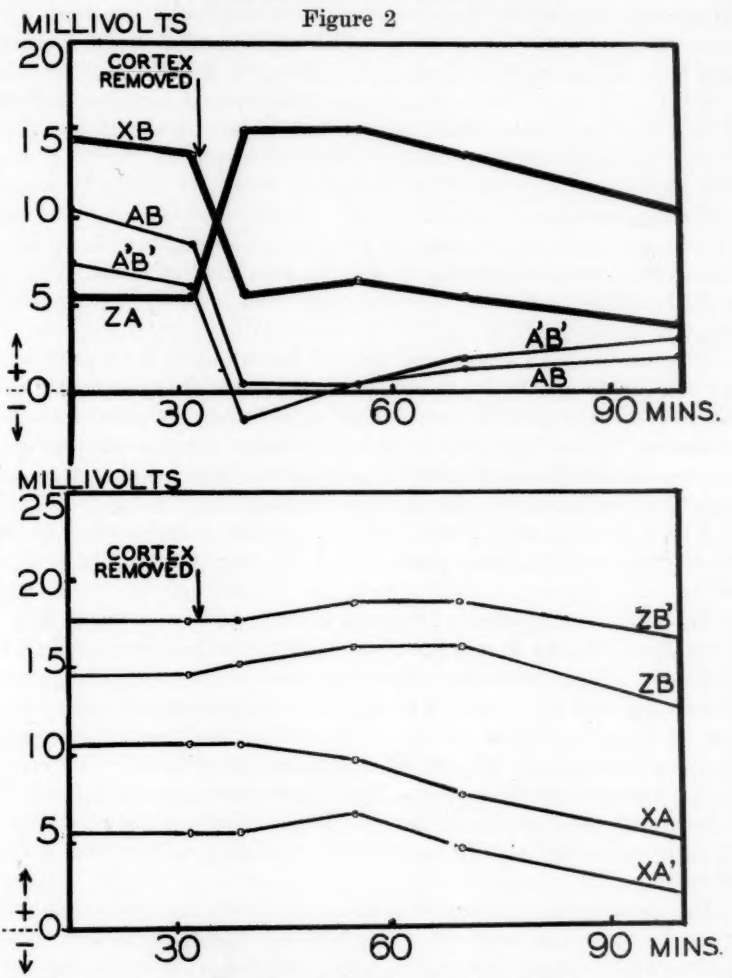

FIG. 2. Upper curves XB, ZA, AB and A′B′ show the relative magnitudes of the E.M.F.'s between the contacts X and B, Z and A, A and B, A′ and B′ respectively, as shown in figure 1.I.

Lower curves ZB′, ZB, XA, and XA′ give the radial potentials of the wood between the correspondingly lettered contacts in figure 1.I. The upper and lower sets of curves are separated in order to avoid confusion. The magnitudes of the E.M.F.'s in the different curves should be compared.

The + or − sign of the potential on the ordinate axis always refers to the observed sign of the potential at the contact designated by the first letter. Thus in curve XB contact X is + to contact B in the external circuit. Similarly in curve A′B′, contact A′ is + to contact B′ in the external circuit, except at one point on the curve where A′ becomes − to B′. This method of designating the orientation of the E.M.F. is used in all the curves of the following figures. Position of arrows indicates the time when the living cortex was removed.

Curves ZB′, ZB, XA and XA′ show that under the conditions of this experiment, no marked effect is produced on the radial potentials of the wood when the cortex is removed. On the other hand curves AB and A′B′ show that the removal of the cortex diminished the E.M.F. between contacts A and B, and A′ and B′, even though these contacts were not disturbed. If the origin of all the E.M.F. between the contacts A and B, and A′ and B′ was located at the contacts then certainly no change in E.M.F. would have been observed by removing the cortex. Since AB and A′B′ are practically duplicates and therefore of the nature of controls for each other we can not regard the result as an accident. Furthermore all similar experiments on other internodes yielded the same kind of result. The drop in the curves AB and A′B′ when the cortex was removed is in my experience only partially, if at all, ever recovered in such an experiment. The interpretation of this result must be that the origin of the E.M.F. in AB and A′B′ is not merely due to a P.D. located at the electrode contacts, but that part of the total E.M.F. originates at loci along the stem between the electrodes. This constitutes important evidence for the validity of the principle of summation of E.M.F.'s in the stem. A permanent drop in the E.M.F. between A and B suggests the conclusion that the cortex is the seat of a permanent E.M.F. which, when the cortex is in position, in some peculiar manner augments (sums with) the longitudinal E.M.F. in the wood axis.

The most striking effect of removal of the cortex is on the E.M.F. between X and B, and Z and A. Note that XB is diminished while ZA is increased. These effects are quite permanent. Since the only difference between XB and ZA is that of an opposite orientation with respect to the polarity of the stem axis, removal of the cortex *must produce its opposite effects by virtue of the opposite orientation of the contacts with respect to the longitudinal axis of the stem.* This apparently means that the E.M.F. in the cortex, when *in situ, opposes* the larger E.M.F. of the wood between the contacts ZA and *augments* (adds to) the E.M.F. of the wood, between the contacts XB.

The experiment reveals the peculiar fact that the cortex is a tissue which determines in part the magnitudes of E.M.F. measured between contacts *on the wood.* The cortex appears therefore to be the seat of an inherent E.M.F. apart from the inherent E.M.F. in the wood. This fact is what we suspected to be true in previous papers but were not able to prove at that time. The above indirect procedure appears now to yield the answer.

For the sake of clearness and unity of thought we shall present at this time a semi-theoretical formulation of the facts. The following experiments will be used as tests for the validity of our interpretation.

The relative magnitudes of the electric polarities and direction of the resulting internal correlation currents are illustrated in the diagram of figure 6. The facts upon which this diagram is based are:

1. A radial E.M.F. with the orientation indicated by the + and − signs exists between center and surface of the wood axis.
2. The magnitude of this radial E.M.F. is less as the apex of the tree is approached. This is arbitrarily indicated by the numbers 8 and 6, which correspond respectively to the numbers of the + and − signs. For the moment we shall limit discussion to only two regions whose location is given by 6, 5 and 8, 2.
3. It will be shown later that there is a radial E.M.F. in the cortex, which is oriented oppositely to that in the wood, that is, the outer surface of the cortex is electropositive in the external circuit to the inner cambial surface. Furthermore this radial E.M.F. *increases* toward the apex of the tree. This fact is indicated by the numbers 5 and 2 in the cortex.

Now suppose we apply contacts at a and b, a current would then flow from a to b in the external circuit. This agrees with all actual experimental observations. Under natural conditions this external "return" circuit we will assume to exist in the conducting outer layers of the cortex. The main seat of E.M.F. is assumed to be in the inner young active phloem and cambium layers similar to the observed location of the greater part of the E.M.F. in the outer layers of the wood (LUND 3). The arrows in the cortex indicate the direction of the internal "return" circuits.

Again suppose we lead off from b and d, the direction of the flow of the current in the internal part of the circuit would be inward in the direction of the vertical arrow. This again is in agreement with experiment. Suppose we make contact at points c and d in the central axis, then the diagram shows that we would expect the current to flow in the *external* circuit from d to c. But now we must remember that since the electrical resistance of the old wood at the center of the tree is very high and the greater part of the E.M.F. designated by the numbers 8 and 6, lies near the surface in the young actively growing wood we would expect to observe a small current, and a small E.M.F. between the central points c and d as measured by the potentiometer. This is just what the experiments in previous papers have shown. The observed magnitude and orientation of the longitudinal E.M.F. in the center of the wood axis depends upon the position of the leads (= inner ends of the glass funnels) in the wood axis. The direction of the arrows at the center indicate the general direction of flow of the correlation current chiefly in that part of the wood having a relatively low electrical resistance.

Suppose we lead off from c and a, experiment shows again that c is electropositive in the external circuit to a, at least in the regions of the

second internode and in all internodes below it (*cf.* later data). Therefore the current *in* the cortex-wood system in such an observation would flow from a to c just as was observed between b and d. However, if we include the E.M.F.'s in both of the polar regions between a and c, and between b and d in a complete system and connect them by conductors, the direction of the current will be determined by the basal region b–d and not by a–c, because the E.M.F. of b–d is 8–2 = 6 units while the E.M.F of a–c is 6–5 = 1 unit. These two are opposed to one another and the resultant E.M.F. will be the difference or 5 units of E.M.F. in our simplified case. The actual direction of flow of electric current in the region between a and c will therefore be that which is indicated by the arrow and not the opposite (*cf.* LUND (3), p. 277).

Regions a–c and b–d we speak of as being electrically correlated. In the above very simple case we may say that the E.M.F. of the region b–d determines more or less the direction of flow of electrical energy in the region a–c. In this manner it now becomes obvious that mutual interaction of E.M.F.'s may result in mutual modification of electrochemical processes and therefore mutual modification of other *linked* metabolic processes in different regions.

I wish to emphasize that the above formulation of the facts is to be thought of as a simplified description of the operation of the internal correlation mechanism. Without such simplification the presentation of the experimental facts becomes difficult and discouragingly indefinite.

Let us now return to the curves of figure 2. The fact that the longitudinal E.M.F.'s A′B′ and AB are reduced by removal of the cortex, can now be understood from figure 6 in the following way. Since the inside surface of the cortex at b is more electropositive (= less electronegative) than the inside surface of the cortex at a, obviously this E.M.F. in the cortex when the latter is in its normal position, operates in *series* with the E.M.F. in the wood. That is, the E.M.F. between any two points in the longitudinal axis of the wood is increased by the presence of the cortex. Removal of the cortex would therefore be expected to result in a decrease in the longitudinal E.M.F. This agrees with the observed result.

The *resultant* E.M.F. of the cortex when in position, is oriented in the same way as the E.M.F. in the region d in the wood. Removal of the cortex should therefore cause a decrease in XB. The curve shows that it does.

On the other hand ZA is increased when the cortex is removed. This must mean that the E.M.F. in the wood in the region c is released from an opposing resultant E.M.F. residing in the cortex.

The opposite effects on XB and ZA caused by removal of the cortex, together with the fact that our diagram fulfills the requirements of observed

experimental facts, suggest very strongly that XB is, to a large extent, a measure of the E.M.F. in the region d–b and that ZA is largely a measure of the E.M.F. in the region a–c. The fact that the E.M.F. between c and d is usually small also suggests the same interpretation.

It appears to be evident from curves ZB′, ZB and XA, XA′ that the E.M.F. in the cortex does not affect these strictly radial potentials in the wood. In our interpretation of the experimental facts we have attempted to construct a diagram of the relations between the orientation and approximate relative magnitude of the internal correlation potentials in wood and cortex, which will fit the facts. The success to which we have attained will in part be indicated by the experiments which follow.

Procedure II

Figure 1.II, curves, figure 3

The arrangement of the experiment indicated by diagram II in figure 1 is essentially the same as that in procedure I, except that another contact Y was added at the center of the wood and the contacts A′ and B′ were dispensed with.

The curves in figure 3 show that most of the potentials underwent a gradual decrease during the period of the experiment. When the drift (shift in flux equilibrium, *cf.* Lund (**6**)) had become uniform the cortex between A and B was removed. The time of removal is indicated by the vertical arrows.

The curves ZB and XA show again that the radial potentials in the exposed wood are affected little if any by removal of the cortex. Note also that ZB > XA. This is to be expected since the position of ZB is more basal than that of XA.

The E.M.F. of XB is decreased and that of ZA is increased by removal of the cortex. This result and its interpretation is again the same as that in procedure I above. It will be noted that this increase and decrease of E.M.F. resulting from removal of the cortex occurs in spite of the continual drift of the E.M.F.'s.

The longitudinal E.M.F. AB also drops to a very low value and remains low or absent during the remainder of the experiment. This result is the same as in procedure I.

The curves for the two measurements YB and YA are interesting for two reasons: First, because YB > YA. This indicates that YB measures largely the radial oblique E.M.F. located toward the basal region near the contact B, while YA measures the corresponding radial oblique E.M.F. in the apical region near the contact A. (*cf.* about p. 638). The second fact of interest is that *after* removal of the cortex, YB and YA *approach each other in magnitude:* Why? The answer is apparently given by our dia-

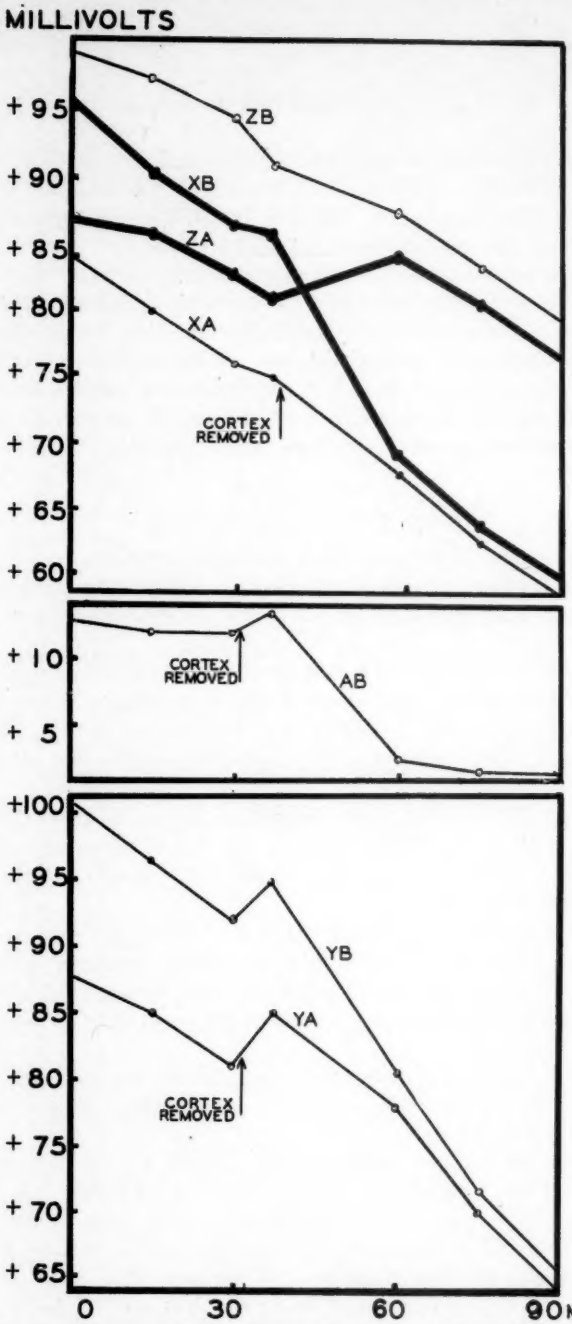

FIG. 3. Upper curves ZB, XB, ZA, and XA refer to E.M.F.'s between corresponding contacts in figure 1.II, and are strictly comparable to the curves in figure 2 designated by the same letters. The middle curve AB is strictly comparable to curve AB in figure 2. Curves YB and YA measure the E.M.F.'s between the corresponding contacts. Their relative magnitudes should be noted.

gram figure 6. Namely, the E.M.F. of the cortex when in contact with the wood, is applied so as to augment the difference in E.M.F. between the regions d and c, namely 8–6 = 2. In order to augment this apparent difference (= difference between YB and YA) the E.M.F. at d(= 8) is increased, while that at c (= 6) is decreased. Or stated in other words the *resultant* E.M.F. of the cortex sums with the local and relatively basal radial E.M.F.'s in the wood since they are oriented in the same direction and are therefore in series, while relatively apical and local radial E.M.F.'s in the wood are decreased because the resultant E.M.F. of the cortex opposes the inherent apical radial E.M.F. in, for example, the region c. A second experiment of this kind gave the same results. Our diagram in figure 6 appears to be in striking agreement with the facts.

Procedure III

Figure 1.III, curves, figure 4

Effect of killing the basal half of the internode by heat.—The second internode from a tree twenty feet high was used. The total length of the internode was 50 cm. The apical and basal diameters of the wood at A and B were 8.5 and 12 mm. respectively. The lengths of the cortex between Y and A, and between Y and B, were 14.5 and 14.0 cm. respectively. The length of each ring of cortex which was removed at A and B was 4 cm. The set up in procedure III was identical with that in procedure II except that the basal half of the internode (stippled) was immersed in boiling tap water for two minutes. The heated cortex assumed a brown color.

During exposure of the basal half of the internode to the boiling water, the apical half was protected from injury by wrapping it in cloths soaked with cool water. The heated half of the internode was now cooled in running tap water, wiped dry and fixed in position with the contacts. The preparation was left for over an hour, during which several readings of P.D. were taken. Toward the end of this period the P.D.'s became relatively constant.

When this had occurred, the dead cortex on the basal half of the internode was quickly removed. The time of removal is indicated by arrows in figure 4. A set of measurements was taken and immediately after these readings, the living cortex on the apical half of the internode was also removed. Readings of E.M.F. were taken thereafter at intervals as indicated on the curves.

The effects produced by removal of the cortex in this type of experiment appear somewhat bewildering at first sight. A study of each curve by itself is however quite illuminating and reveals a number of interesting facts. The first fact which is apparently indicated in the curves is that

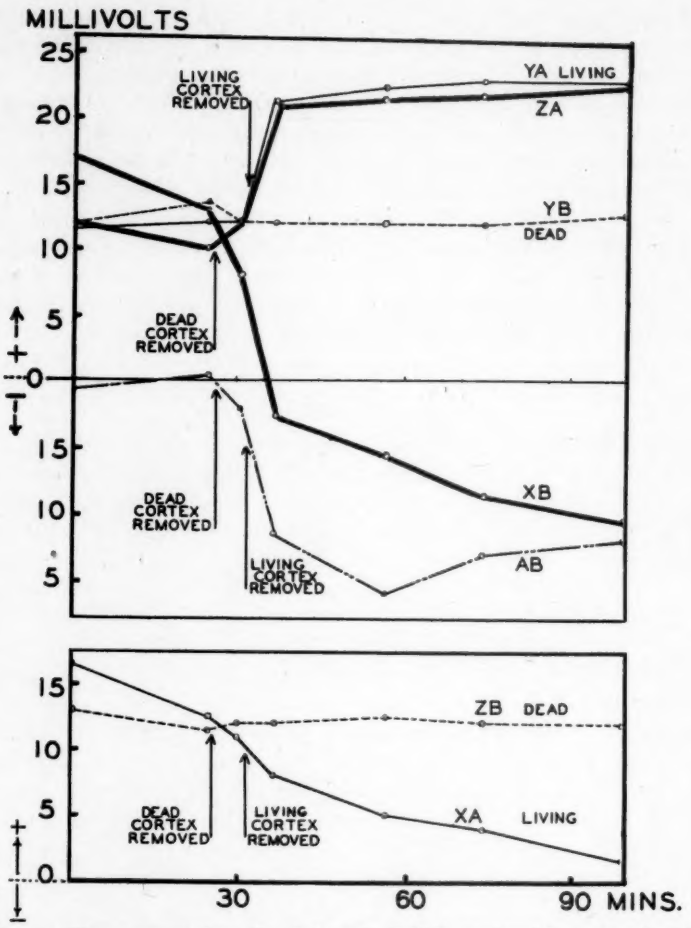

FIG. 4. Upper curves show the changes in E.M.F. produced by removal of basal (dead) and apical (living) cortex between the different pairs of contacts in figure 1.III. Lower curves are drawn on separate ordinates to avoid confusion. For interpretation see text.

removal of the living apical half of the cortex produces quicker and greater change in certain E.M.F.'s than the removal of the dead cortex.

Curve ZB which represents the radial E.M.F. in the dead wood is constant and has the same orientation as the E.M.F. in the living tissue. Similarly YB has the same orientation but not a greater magnitude relative to YA in the living apical half, as would have been found if the region YB

were living.[1] Curve XA of the radial E.M.F. of the living wood shows a drift in the E.M.F. but is apparently not affected by removal of the cortex. This result agrees with those of all other experiments.

Comparing YA and YB we see that YA which is living is increased by removal of the living cortex just like ZA was increased in procedures I and II above. On the other hand YB, which is dead, is not at all affected by removal of its dead cortex. Evidently ZA is comparable to YA since both include the living region and are increased by an equal amount. We may perhaps infer from this that YA and ZA measure the E.M.F. of approximately the same region, namely that near A. On the other hand the contacts X and Y with respect to B are not the same, because XB includes the living wood end and is therefore affected by removal of the living cortex, while YB does not include any living wood and is not affected by removal of either living or dead cortex. This is the reason why removal of the cortex does affect XB but does not affect YB. The E.M.F. of XB is decreased in a similar way to that in procedures I and II. However its polarity is finally inverted so that X becomes electronegative to B.

Removal of the living cortex decreases very much (= inverts) the longitudinal wood E.M.F., AB. This is fundamentally the same result as that obtained in all experiments of procedures I and II above. With reference to figure 6, the interpretations of the effects of removal of the living cortex in procedure III are identical with the interpretations given above for procedures I and II, and therefore we shall not repeat them.

Procedure IV

Figure 1.IV, curves, figure 5

Effect of killing the apical half of the internode by heat.—The experimental procedure was the same as that in the previous experiment except that the third internode of the same tree was used, and the apical half of the internode was killed by immersion in boiling water for four minutes. The times of removal of the dead and living cortex are indicated by the vertical arrows on the curves.

[1] Any partial critic of the oxidation-reduction theory of the origin of continuous bioelectric currents might obviously consider these facts as support for the common theories of bioelectric currents, according to which bioelectric E.M.F.'s are due to differences in concentration of inorganic ions. In this connection the writer merely wishes to repeat that several types of electric potentials in all probability enter into various bioelectric phenomena. The problem which concerns us is the identification of the particular kinds of E.M.F. which occur in different electrical phenomena inherent in the living cell. In any case the existence of a P.D. in a dead system is obviously no secure basis for inferences regarding the nature of the E.M.F.'s in the previous living state of that system.

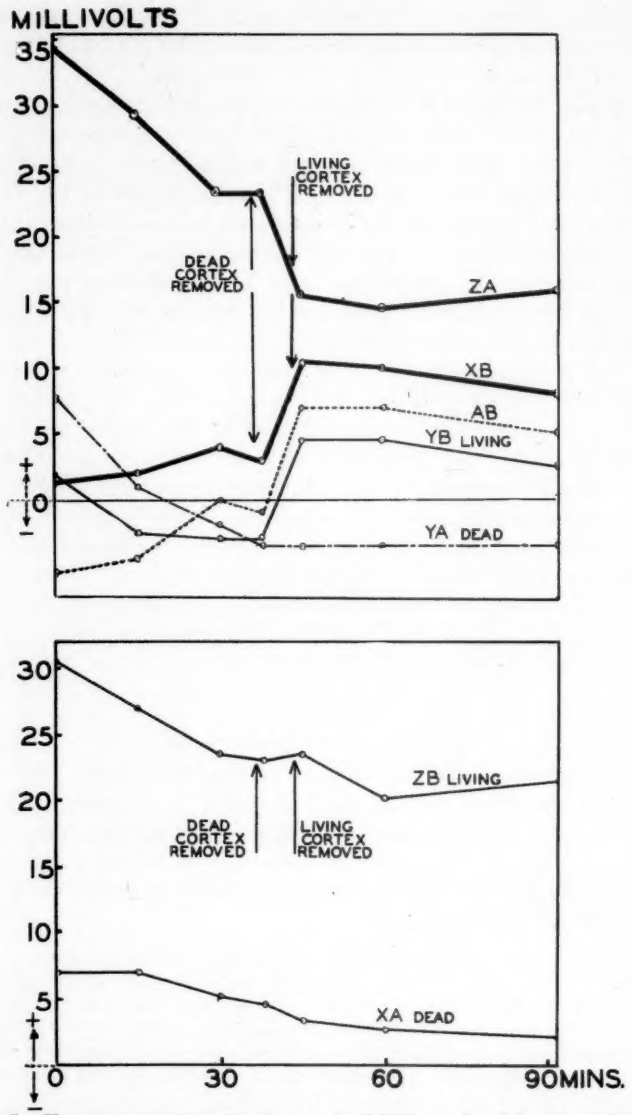

FIG. 5. Upper curves show the changes in E.M.F. produced by removal of apical (dead) and basal (living) cortex between the different pairs of contacts in figure 1.IV. The corresponding curves in figues 4 and 5 should be compared. For interpretation see text.

Again the radial E.M.F.'s, ZB and XA do not show any appreciable affect of removal of the cortex. The longitudinal wood E.M.F., AB, now shows a distinct increase in E.M.F. as compared to a *decrease* in all the previous types of experiment when the living cortex was removed. The question is, why do we obtain opposite effects on AB in the two procedures III and IV when the living cortex is removed?

Referring to figure 6 we recall that the *resultant* of the E.M.F.'s in the cortex is oriented in such a manner that it *augments* (= increases) the radial E.M.F. of the wood in the region d, but *opposes* the radial E.M.F. of the wood in the region c. *After* the removal of the living cortex in procedure III, A is electronegative to B (inverted polarity). *In this case* the resultant E.M.F. of the cortex is evidently opposed to the inherent longitudinal E.M.F. of the wood when the cortex is *in situ*. The result is that the inherent (inverted) longitudinal E.M.F. in the wood between A and B in curve AB of figure 4 is diminished by presence of the cortex and increased by its removal.

The opposite explanation evidently applies to curve AB in procedure IV because the curve shows that *after* removal of the living cortex, A is electropositive to B. In this case the orientation of the resultant E.M.F. in the cortex was again opposite to that in the wood, since removal of the living cortex in procedure IV resulted in an increase in the longitudinal E.M.F. of the wood. Since contacts Z and A of procedure III are comparable respectively to contacts X and B of procedure IV, in their relation to dead and living parts of the stem, we should expect that removal of the living cortex would affect both of these E.M.F.'s in the same manner. The same statement applies to curve ZA of figure 5 and curve XB of figure 4. Both of these expectations are fulfilled as shown by the curves.

Again since the contacts Y and A in procedure III are comparable respectively to contacts Y and B in procedure IV in their relation to dead and living parts, we should perhaps expect that removal of the living cortex would effect both of these E.M.F.'s alike. The curves show that both of these E.M.F.'s are increased. Finally curve YA shows that the E.M.F. of this dead segment is not affected by removal of the dead cortex. This result is the same as that in curve YB of figure 4, procedure III.

Discussion

Increase in the radial E.M.F. in the cortex toward the apex

The preceding experiments, especially those of procedures I and II, have shown that the effects of removal of the cortex[2] lead to the conclusion

[2] By cortex is of course meant the living parts without specifying which particular cells are primarily concerned in the electric phenomena. Identification of the particular cells concerned is of course a problem for the future.

that the resultant E.M.F. of the cortex augments the resultant longitudinal E.M.F. of the wood. This leads to the inference that the radial E.M.F. in an apical part of the cortex is relatively greater than that in a more basal part of the cortex, at least under conditions of absence of injury and stimulation. The arbitrary numbers 5 and 2 in figure 6 are intended to indicate this fact.

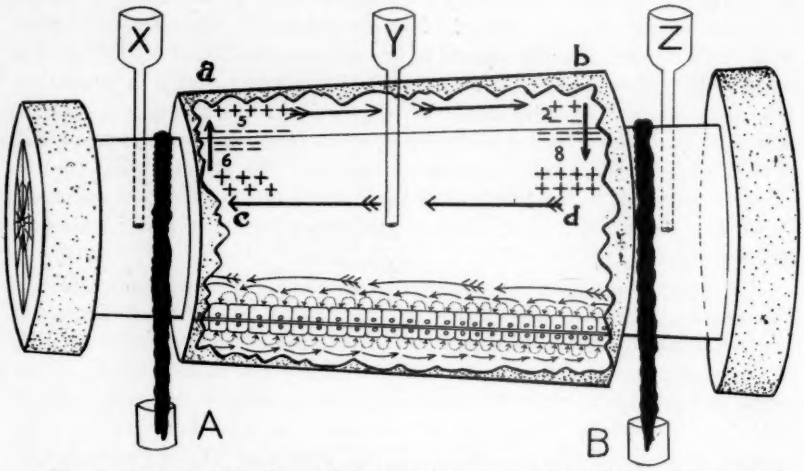

FIG. 6. Diagram of an internode having the same contacts as that shown in fig. 1, procedure II. The numbers 2 and 5 represent arbitrarily chosen values of the radial E.M.F.'s in the cortex at this level, and correspond to the numbers of + and − signs. Similarly the numbers 8 and 6 represent arbitrarily chosen values of the radial E.M.F. in the wood. The indicated orientation and relative magnitudes of the radial E.M.F.'s in cortex and wood are those which would fulfill all the requirements of the observed experimental facts. The direction of flow of the resulting current produced by the two systems of E.M.F.'s in cortex and wood is indicated by the direction of the arrows. Note that the resultant E.M.F. in the cortex is in series with that in the wood. At the bottom of the figure is shown two layers of cells in section; these represent diagrammatically the bipolar cambium and other living cells in cortex and wood in which the E.M.F.'s originate. The direction of the arrows indicate the general direction of the flow of electric current. For fuller description see text.

Now if the reader will refer to the diagram E in figure 7, page 271, and table 4, page 275 in the article by LUND (3), it will be found that the magnitude of the basal-apical oblique E.M.F.'s have always the following relation $E_3 > E_2 > E_1$. A specific illustration is given in table 4 where $E_3 = 67.5$ m.v., $E_2 = 52.0$ m.v., and $E_1 = 31.5$ m.v. As the external contact in such a series of measurements is moved farther toward the apex the E.M.F.'s decrease until they finally become zero. Above this zero point the E.M.F.'s

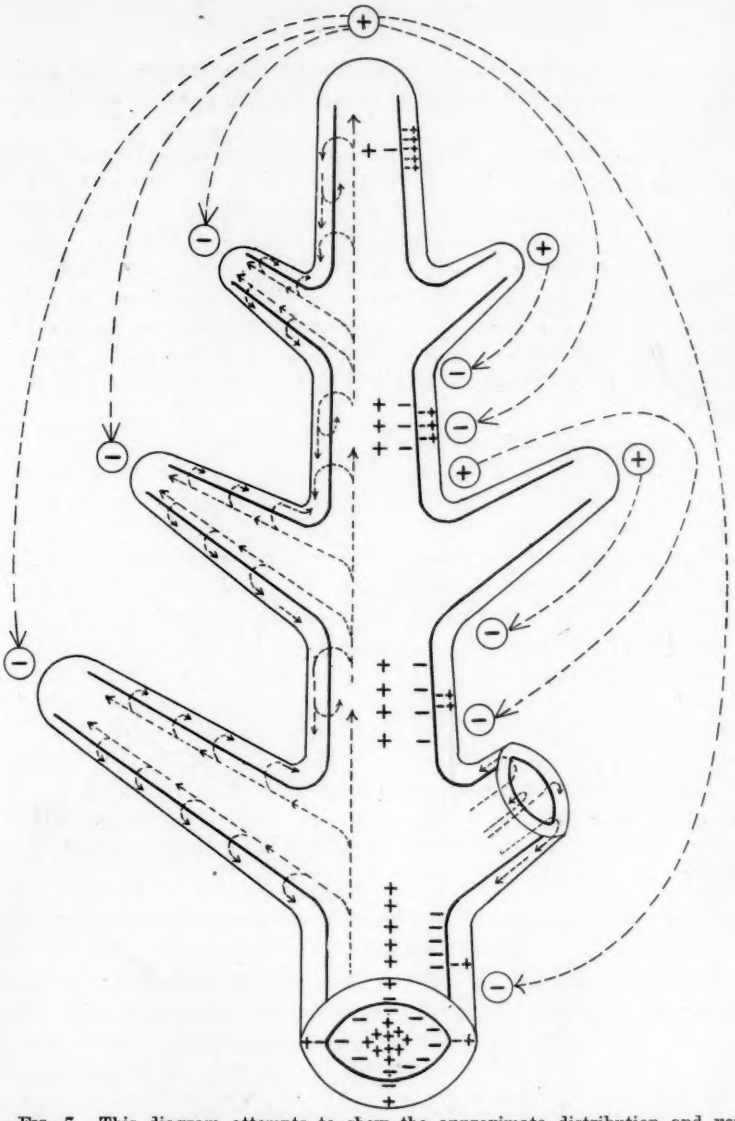

FIG. 7. This diagram attempts to show the approximate distribution and usual orientation of the E.M.F.'s in the wood and cortex of the Douglas fir together with the resulting orientation of the externally observed polarities in the main axis and branches. The relative magnitude of the E.M.F.'s in wood and cortex of the main axis are indicated by the groups of + and − signs on the right hand side of the diagram. Each branch is the seat of a similar distribution of E.M.F.'s.

The system of arrows in the wood and cortex on the left hand side indicates the direction of the resultant E.M.F. in the wood-cortex system in the tree under conditions of absence of stimulation. Experiments show that the E.M.F.'s in the apical regions are especially subject to large fluctuations in magnitude and direction. The diagram is of course not to be taken too literally, although it fits all the observed experimental facts which are known at present.

increase again but the outside apical contact now becomes electropositive (in the external circuit) instead of electronegative to the lower contact in the center of the wood axis.

A series of measurements was carried out on the apical ends of lateral branches. The contacts were those designated by GA, GC, GB, GD, GE and GF in figure 1 of the preceding paper (LUND 5). All of these measurements showed that the apical contacts were electropositive to G except F, and GF exhibited only a small E.M.F. This is interpreted to mean that the total radial E.M.F. of the wood plus cortex diminishes toward the apex. This decrease is due to the decrease in diameter of the wood axis toward the apex, because it has been shown that the radial E.M.F. is proportional to the thickness of the peripheral living active part of the wood axis.

The basal-apical oblique E.M.F.'s in apexes are relatively large. The greater part of this E.M.F. must have its origin in the cortex since the orientation of the E.M.F. between these contacts is reversed in the extreme apical regions. In other words the radial and therefore the resultant longitudinal E.M.F. in the cortex of the growing apical segment is greater than that in the thin tubular wood axis. It is evident that in the apex it is the E.M.F. of the cortex which dominates the electromotive phenomena.

In figure 7 this basal-apical increase in radial E.M.F. in the cortex is indicated by the larger number of + and – signs. We conclude from the preceding facts that there must exist a point at some distance below the apex where the radial E.M.F. of the wood plus cortex equals zero.[3] The facts taken as a whole show that the radial E.M.F. of the apex is subject to unusually large fluctuations in magnitude when compared to other regions of the tree. The person who likes to contemplate the adaptive features of biological mechanisms will find interesting material in the operation of the mechanism of electric correlation.

GENERAL CONSIDERATION OF THE RESULTS AND THEIR BEARING ON ENERGY TRANSFORMATION IN THE PLANT

The experiments have revealed a group of new phenomena, the consequences of which are of special interest for cell correlation and related processes. In the first place they show in an unequivocal manner that the E.M.F. which is observed when we measure the external electric polarity of the stem and branches of the tree is the algebraic result of a system of internal E.M.F.'s, the elements of which are located in two main regions, namely the cortex and wood. The experiments show that the *resultants* of these systems of E.M.F.'s augment one another, that is to say they operate

[3] This point corresponds to that on the main axis of the stem at which the extrapolated curves of E.M.F. in LUND ((3) figure 4, page 265) cuts the horizontal axis.

in series like two complex systems of batteries. In general they show that an electric current flows downward in the outer cortex and upward in the wood axis. The results therefore confirm previous observations but in addition show that the cortex is the seat of a separate E.M.F. Figure 7 is presented as an approximate summary of the facts to date. To help visualize a little more accurately the direction of flow of the transverse and longitudinal components of the currents, the bipolar cambium and other bipolar origins of the E.M.F. are represented diagramatically in figure 6 by two layers of cells with a system of arrows to indicate the general direction of the correlation currents. Future experimental analysis of the distribution of correlation currents will of course be toward a description of the patterns in microscopic dimensions. In all probability these patterns will be found to correspond more or less to the complex cellular architecture of the wood and cortex in which they originate and in which their energy is being transformed and dissipated.

One fact of extreme interest which must be clearly recognized is that when we measure the E.M.F. between any two contacts the magnitude of this E.M.F. is in general not necessarily directly proportional to the total electric current which flows in the local circuits in the tissues between the electrode contacts. In other words the system operates more like a complex network of conductors with a relatively fixed pattern of local origins of E.M.F.'s. It appears certain from this consideration that relatively large amounts of electrical energy are being transformed into electrical work and heat, which would not be indicated by single measurements of E.M.F.'s or electric currents led off from a single pair of contacts as we do in actual practice.

It is not improbable that a considerable part of the total heat energy output by the plant is derived from continuous electric currents, the energy of which appears only secondarily as heat. If this is true then it is obvious that the experiments have opened to us a new vista of the phenomena of energy transformations in the plant. We shall discuss briefly some of the possibilities in what follows.

CONTINUOUS CORRELATION CURRENTS AS THE POSSIBLE SOURCE OF ENERGY FOR ELECTROENDOSMOTIC TRANSPORT IN THE PLANT

The writer can not forego the opportunity at this time of presenting what appears to him a distinct possibility, namely that one of the functions of the continuous electric current which is directed upward in the wood is to supply electrical energy for electroendosmotic flow of sap in an upward direction in the conducting vessels of the wood. This suggestion may also apply to a downward flow in the cortex as well as transport across the stem.

If this is the case we would expect that the well known equation for velocity of flow in a system of capillaries caused by the application of an E.M.F. would apply, namely,

$$v = \frac{q \varepsilon H \zeta}{4 \pi \eta l}$$

In this equation v = volume flow, q = area of cross-section of the capillary system, H = the applied E.M.F., ε = the dielectric constant of the liquid, η = the viscosity, and l = length of the capillary system, ζ = the electrokinetic potential between the liquid and wall of the capillary. Suppose we consider a certain bundle of conducting vessels or a single conducting vessel in the water conducting region of the wood (OVERTON **9**). The orientation of the radial and resultant longitudinal E.M.F.'s is that represented in the diagram figure 6. It is evident that q, ε, η and l are determinable quantities. H is supplied by the inherent E.M.F., leaving the electrokinetic potential ζ to be considered. Now it has been shown by STAMM (**10**) that the orientation of the electrokinetic potential of the walls of wood vessels against water is such that the liquid in all the types of wood which he examined namely, Sitka spruce, Alaska cedar, western red cedar, western hemlock, Douglas fir and yellow poplar, moved from anode to cathode, showing definitely that water with respect to the capillary vessel wall carries a positive charge.

STAMM'S experiments therefore demonstrate first, that an electrokinetic potential exists between the water and the wall of the conducting vessel. The presence of the electrokinetic potential as the equation shows, is a *sine qua non* for the occurrence of electrokinetic flow, for if ζ is 0 then v = 0. The second significant fact about STAMM'S experiments is that the electrokinetic potential is oriented in the right direction, that is, water is electropositive to the wall of the vessel and therefore the observed inherent E.M.F. in the wood, figure 6, which now takes the place of H in the equation will of necessity tend to transport the water upward and not downward in the vessels of the wood.

With these facts in mind a few preliminary experiments were carried out on the apexes of lateral branches of the Douglas fir. The procedure was as follows. The apical part of a lateral branch was cut off about one inch below the origin of the two most distal symmetrically placed lateral shoots. Care was taken to select tips of branches in which these two lateral shoots were placed exactly opposite one another at the base of the main axis shoot. The cut basal end was dipped into a solution of eosin in water. Tap water electrode contacts were attached to the tips of the two lateral shoots. A constant electric current of a few microamperes was sent into the apex of one of the symmetrical lateral shoots and out through

that of the other. The applied current therefore passed downward in one and upward in the other shoot. The current was left on usually for eight to twelve hours. During this period the solution usually ascended several cm. in the wood axis.

Removal of the cortex showed that in most cases the distance to which the dye had risen in the two lateral shoots was unequal. In all the experiments showing distinct inequality the solution had penetrated to the greatest distance in that lateral shoot in which the electric current flowed from base to apex in the wood. In some of these preliminary experiments the difference was very marked while in others the result remained uncertain.

The results of these preliminary experiments are merely to be considered as suggestive. However, the results indicate agreement with the interpretation which we have presented above.

At present the phenomena are being investigated with a fuller consideration of the many factors which are involved. It is obvious that such phenomena as guttation and bleeding pressures may be the expression of the same type of mechanism.

The possible application of correlation potentials in roots (LUND and KENYON, **6**) to the problem of differential absorption of ions will be considered in later papers.

Summary

1. The results of the experiments constitute direct and conclusive evidence that the principle of summation of E.M.F.'s applies to phenomena of electric correlation between living cells in the Douglas fir.

2. The cortex of the Douglas fir is the origin of a characteristic E.M.F. The orientation of the radial E.M.F. in the cortex is opposite to that in the wood.

3. Several lines of evidence show that this radial E.M.F. in the cortex increases toward the apex, while that in the wood decreases.

4. It is shown that the resultants of each one of the two systems of E.M.F.'s in the cortex and wood operate as if they are placed in series.

5. Removal of a ring of living cortex which lies between two contacts placed on or in the wood axis results in a permanent change in the previously observed E.M.F. between these contacts. The direction of change depends upon the position of the contacts.

6. Removal of similar rings of cortex from stems which had been killed by heat did not produce any such marked effects.

7. Electromotive forces are present in stems killed by heat, but the behavior of such E.M.F.'s is radically different from those in the living cortex.

8. Attention is called to the possible rôle under certain conditions of electric correlation currents in the wood as a source of energy for electro-endosmotic transport of water upward in the conducting vessels of the wood. It is shown that all present known facts agree with this possibility.

9. The observed small quantities of electric energy which may be led off from two contacts on the tree are not to be considered as indicative of the actual amounts of electrical energy which are transformed into work and heat in the plant. There is every reason to believe that the quantity of electrical energy which is transformed is much larger than has heretofore been suspected.

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LITERATURE CITED

1. LUND, E. J. Electric polarity in the Douglas fir. Pub. Puget Sound Biol. Sta. **7**: 1–28. 1929.
2. ————. The relative electrical dominance of growing points in the Douglas fir. Pub. Puget Sound Biol. Sta. **7**: 29–37. 1929.
3. ————. Internal distribution of the electric correlation potentials in the Douglas fir. Pub. Puget Sound Biol. Sta. **7**: 259–287. 1930.
4. ————. Relation between continuous bioelectric currents and cell respiration. II. Jour. Exp. Zool. **51**: 265–290. 1928.
5. ————. External polarity potentials in the apex of the Douglas fir before and after mechanical stimulation. Plant Physiol. **6**: 507–517. 1931.
6. ———— and KENYON, W. A. Relation between continuous bioelectric currents and cells respiration. I. Electric correlation potentials in growing root tips. Jour. Exp. Zool. **48**: 333–357. 1927.
7. MARSH, G. Relation between continuous bioelectric currents and cell respiration. IV. The origin of electric polarity in the onion root. Jour. Exp. Zool. **51**: 309–325. 1928.
8. ————The effect of applied electric currents on inherent cellular E.M.F. and its possible significance in cell correlation. Protoplasma **11**: 447–474. 1930.
9. OVERTON, J. B. Seasonal variations in the hydrostatic-pneumatic system of certain trees. Amer. Jour. Bot. **17**: 1043. 1930.
10. STAMM, ALFRED J. Electroendosmose through wood membranes. Colloid Symp. Monographs **4**: 246–257. 1926.

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NOTES ON THE NUTRIENT REQUIREMENTS AND THE HISTOLOGY OF THE CRANBERRY (*VACCINIUM MACROCARPON* AIT.) WITH SPECIAL REFERENCE TO MYCORRHIZA[1]

RUTH M. ADDOMS AND F. C. MOUNCE

(WITH THREE FIGURES AND TWO PLATES)

Introduction

The nutrition of the cranberry, as well as of various other members of the Ericaceae, is complicated by the fact that under ordinary cultural conditions the green plant exists in close association with a fungus. Although an association of a fungus with the roots of a higher plant is called a mycorrhiza, the literal definition of the word must be extended somewhat to include the condition that exists in the Ericaceae, for in this family the fungus is not confined to the roots, but is coextensive with the growth of the plant, occurring in practically every organ. The fungi associated with the Ericaceae were named by TERNETZ (**14**) in 1907 as strains of *Phoma radicis*, and have since been isolated by RAYNER (**9**) and other workers. The most complete summary of the work that was done on mycorrhiza by various workers prior to 1927 is to be found in a monograph by RAYNER (**10**).

Field experiments by various workers (**1, 12, 15**) have led to conflicting reports in regard to the value of various nitrogenous fertilizers. It is possible that the presence of the endophytic fungus may make available forms of nitrogen that could not otherwise be utilized by the green plant. The present project was undertaken in an attempt to contribute to our knowledge of the fertilizer requirements of the cranberry, and of the structural and nutritional relationships between the cranberry and its endophyte. This paper represents only a preliminary report; it is hoped that a continuation of the work will yield further results.

Methods

CULTURAL

All of the plants used in these experiments were of the variety Early Black.[2] On April 26, 1930, several hundred rooted cuttings selected for uniformity were removed from bog soil, washed clean, transplanted to washed quartz sand in new eight-inch flower pots, two to four plants to a

[1] Journal Series paper of the New Jersey Agricultural Experiment Station.

[2] The plants were obtained through the courtesy of MISS ELIZABETH C. WHITE, of Whitesbog, N. J.

pot, and placed in a greenhouse. The stems of these plants (fig. 1) averaged from 13 to 15 cm. in length. For a period of three weeks, during which time they resumed growth after transplantation, they received a full nutri-

FIG. 1. Rooted cuttings, as transplanted to sand culture on April 26, 1930. $\times \frac{1}{4}$.

ent solution (see Series 2, below). On May 17 they were divided into 4 series, which from then until the latter part of August received the following nutrient treatments:

SERIES 1.—21 pots formed a culture triangle, each pot receiving a different proportion of $MgSO_4$, KH_2PO_4, and $Ca(NO_3)_2$ as shown in table I, but all having the same total osmotic concentration (**6**). The salts were supplied by SHIVE'S constant-drip method (**13**). This series was run in duplicate.

SERIES 2.—28 pots received solution R_2S_4 of the above triangle, a solution which had been shown by previous experiments to be well-suited to the growth of many kinds of plants. Nitrogen was supplied as $Ca(NO_3)_2$.

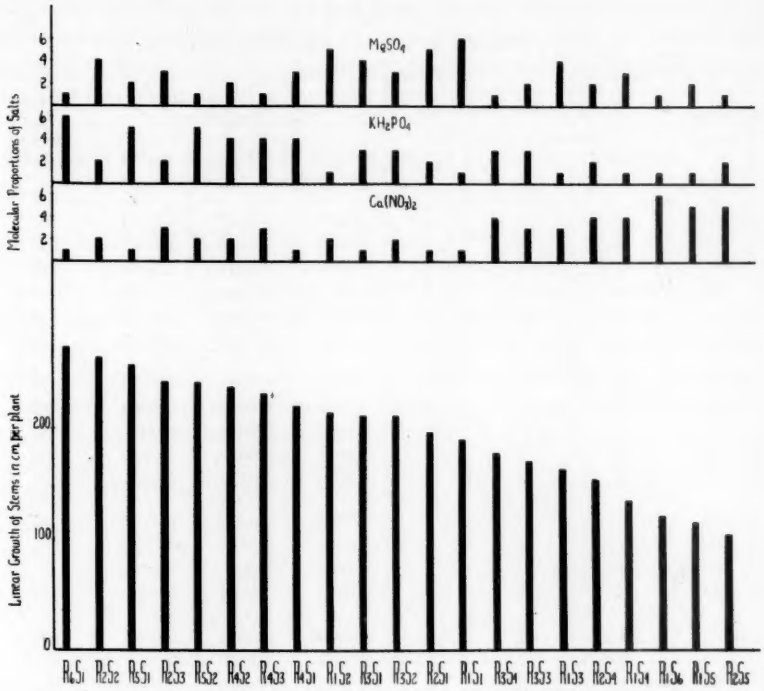
Molecular Proportions of Salts
$MgSO_4$
KH_2PO_4
$Ca(NO_3)_2$
Linear Growth of Stems in cm. per plant
0
100
200
R_4S_1 R_2S_2 R_5S_1 R_2S_3 R_5S_2 R_4S_2 R_4S_3 R_4S_1 R_1S_2 R_3S_1 R_3S_2 R_2S_1 R_1S_1 R_3S_4 R_3S_3 R_1S_3 R_2S_4 R_1S_4 R_1S_6 R_1S_5 R_2S_5

FIG. 2. Below, block diagram showing the amount of linear growth of stems in each culture of the triangle. Above, block diagrams showing the molecular proportions of $MgSO_4$, KH_2PO_4, and $Ca(NO_3)_2$, respectively, in each of the nutrient solutions of the triangle.

SERIES 3.—28 pots received a solution entirely lacking in nitrogen. Solution R_2S_4 was modified by the substitution of $CaCl_2$ for $Ca(NO_3)_2$. From time to time the sand was tested for nitrates, always with negative results.

SERIES 4.—6 pots received a solution of the following composition (5) in which nitrogen was supplied as ammonium sulphate:

SALTS USED	PARTIAL VOLUME-MOLECULAR CONCENTRATION
$(NH_4)_2SO_4$	0.0014
KH_2PO_4	0.0020
$CaCl_2$	0.0073
$MgSO_4$	0.0071

TABLE I

COMPOSITION OF CULTURE SOLUTIONS USED

SOLUTION NUMBER	MOLECULAR PROPORTIONS			PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			AVERAGE STEM GROWTH PER PLANT
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	
							cm.
1R1S1	1	1	6	0.0027	0.0027	0.6161	190
S2	1	2	5	0.0025	0.0049	0.0123	213
S3	1	3	4	0.0024	0.0071	0.0094	163
S4	1	4	3	0.0022	0.0089	0.0067	136
S5	1	5	2	0.0022	0.0108	0.0043	116
S6	1	6	1	0.0020	0.0122	0.0020	121
R2S1	2	1	5	0.0053	0.0027	0.0132	197
S2	2	2	4	0.0049	0.0049	0.0099	263
S3	2	3	3	0.0047	0.0071	0.0071	242
S4	2	4	2	0.0045	0.0090	0.0045	155
S5	2	5	1	0.0041	0.0104	0.0021	103
R3S1	3	1	4	0.0076	0.0025	0.0101	211
S2	3	2	3	0.0072	0.0048	0.0072	211
S3	3	3	2	0.0068	0.0068	0.0045	171
S4	3	4	1	0.0065	0.0086	0.0021	178
R4S1	4	1	3	0.0099	0.0025	0.0074	220
S2	4	2	2	0.0094	0.0047	0.0047	238
S3	4	3	1	0.0090	0.0068	0.0022	231
R5S1	5	1	2	0.0123	0.0024	0.0049	257
S2	5	2	1	0.0118	0.0047	0.0023	241
R6S1	6	1	1	0.0145	0.0024	0.0024	272

This series was started about four weeks later than the others.

In all of the cultures the total osmotic concentration was approximately one atmosphere. Iron was supplied from time to time as ferrous sulphate. The acidity of the various nutrient solutions ranged from pH 4.9 to pH 5.6, a range somewhat less acid than that of most cranberry bogs.

Supplementary experiments, carried on from time to time as need arose, are described later in this paper.

HISTOLOGICAL AND MICROCHEMICAL

Cranberry plants from the cultures described above, and also plants of the same variety from a commercial bog were examined microscopically for details of structure of the cranberry plant and of its endophytic fungus.

Microchemical tests were in general those suggested by Dr. ECKERSON (3) of the Boyce-Thompson Institute. For the most part it was found desirable to study fresh material, colored by means of ruthenium red, aniline blue, mercurochrome, or other stains. For fixed material both orange G and safranin were found useful when sufficiently dilute.

Results

CULTURAL

All of the plants, with the exception of those that received no nitrogen, and the three or four poorest cultures of the triangle, were fairly green, grew vigorously, and produced runner-growth greatly exceeding that of similar plants that were grown for the same period in a commercial cranberry bog. In fact, the plants would be regarded by a commercial cranberry grower as too vegetative for the production of fruit. The linear growth of stems of the various cultures in the triangle is shown in table I as average growth per plant in centimeters. Correlation between amount of stem-growth and composition of nutrient solution is shown graphically in fig. 2. The linear growth of stems in Series 2 to 4 is given below:

SERIES	LINEAR GROWTH OF STEMS (AVERAGE OF 4 PLANTS) IN CM. PER PLANT
Series 2, nitrate (R_2S_4)	227
" 3, minus nitrogen	52
" 4, ammonium	370

Fig. 3 shows the general appearance of representative plants on August 22, and gives indication of the vigor of their growth. With the exception of the minus-nitrogen series and of the plants that received the highest concentrations of nitrate, all plants produced large and apparently healthy root systems.

Bog soil, the usual habitat of cranberry plants, has a high content of humus and a relatively low content of nitrates. Because of these facts, an additional experiment was conducted. Five grams of dried blood, which is often used as a fertilizer in cranberry bogs, and which has a high content of organic nitrogen, were added to each of two pots of cranberry plants that were receiving the minus-nitrogen solution (Series 3). The plants responded quickly to the treatment, developed in apparently healthy manner, and produced runner-growth comparable to that of the ammonium series (Series 4). After these cultures had been continued for several weeks, the sand was tested for nitrates, ammonia, and amino acids. The diphenyl-

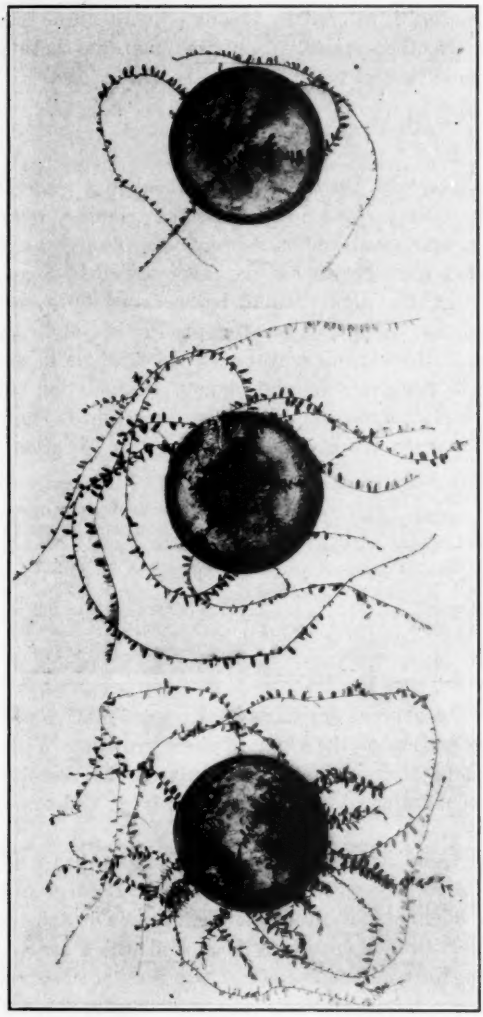

FIG. 3. Representative plants as they appeared on August 22, 1930. Top, culture R_1S_3 (high nitrate). Middle, culture R_2S_2 (low nitrate). Bottom, ammonium culture. $\times 1/10$.

amine test indicated the presence of only a faint trace of nitrates; aspiration yielded ammonia; SORENSEN'S formal titration method showed a considerable quantity of amino acids.

It thus seems possible, although nothing has been proved, that the cranberry plant or its endophyte or the combination of the two (mycorrhiza) can utilize amino acids. Additional evidence is furnished by the results of an experiment performed during the latter part of August. Three pots of minus-nitrogen plants (Series 3) were selected for uniformity; one was supplied with a solution containing nitrogen in the form of nitrate only (solution R_2S_4), one in the form of ammonium (solution of Series 4), and one in the form of amino acids only (glycine, leucine, tyrosine, and asparagine in a total concentration of 0.1 per cent.). All solutions were supplied by SHIVE'S constant-drip method. The amino acid solution was boiled each day before it was added to the cultures, to minimize bacterial action. The sand was tested frequently for nitrates, always with negative results; it did, however, contain a trace of ammonia. The plants receiving ammonium and amino nitrogen respectively responded much more quickly than those receiving nitrate nitrogen, and within a week showed appreciable growth.

HISTOLOGICAL AND MICROCHEMICAL

The roots of the cranberry plant, in common with those of many other members of the Ericaceae, are very fine and much branched, with no root hairs, so that nutrients must be absorbed by unelongated epidermal cells or must enter those cells through the mycelium with which they are infested. Mycorrhiza was observed in all the culture series of these experiments as well as in plants that grew for the same period in a bog. The bog plants, however, were much more heavily infected than any of the sand cultures. The minus-nitrogen series in general showed least infection, although a few individual plants in this series were heavily infected.

The mycelium forms a branching mass over the surface of the young roots (plate XI, fig. 4), extending practically to the tip. Young hyphae are slender and colorless, older ones larger and brown. Branches of the mycelium penetrate epidermal cells, and within these cells form intertwined masses, as shown in plate XI, fig. 5.

In general the root is similar to that described by JANSE (**4**) for other species of *Vaccinium*. The internal structure is most readily observed in cross-section (plate XII, fig. 8). Beneath the epidermis, which consists of conspicuously large cells, lies a single layer of cortical parenchyma, and beneath this, the endodermis, which becomes thick-walled and suberized at an early age. The pericycle is one or two cells thick, and the protoxylem points are usually three in number, although there is some variation. My-

celium is plainly visible in the layer of cortical parenchyma, although its form is not so clear in fixed as in fresh material. Partial digestion of the mycelium may occur, resulting in the appearance in fresh material of granular masses rather than of distinct hyphae. Mycelium has not been observed in the endodermis or in the stele of young roots; this finding is in accordance with that of RAYNER (**10**) for *Calluna*. In older woody roots, however, it has been observed by both Dr. ECKERSON and the present authors in the parenchyma of xylem and phloem.

Stems of the plants that had received different cultural treatments showed greater differences than the roots. The minus-nitrogen (Series 3) plants grew so slowly that a relatively small portion of each stem was succulent, a relatively large portion woody and stored with starch. Such an accumulation of carbohydrates is not peculiar to the cranberry, but represents the usual condition in plants in which vegetative growth is curbed by deficiency of nitrogen or of certain other elements. Also, the plants of Series 1 that received the highest concentrations of nitrate grew slowly and were high in carbohydrates. Otherwise the plants of the various series showed no significant differences in stem structure.

The structure of a portion of a young stem is shown in plate XI, fig. 7. Strands of primary xylem are numerous, and cambial activity results in the formation of a continuous vascular cylinder, interrupted by uniseriate rays. Just outside the sieve-tubes and companion cells of the phloem are several layers of thin-walled parenchyma and several layers of angular cells that gradually lengthen and become fibers, although never extremely thick-walled. These layers of parenchyma and fibers probably represent pericycle, although since an endodermis is not clearly distinguishable there is no demarcation between stele and cortex. The several layers of thin-walled cortical cells, some of which contain an anthocyanin pigment, are bounded by an epidermis that is rich in oil and is heavily cutinized. In the young stem, chloroplasts are located chiefly in cortical parenchyma and in medullary rays. Calcium oxalate crystals are common in cortical parenchyma, especially at the nodes, in the basal portion of axillary buds, and in the apical meristem.

As the stem grows older, the outer regions are split off as a result of the activity of a cork cambium that is formed in the parenchyma directly external to the phloem. Thus the fibers become the outermost layer, and as expansion continues they too are crushed and split off, and their place is occupied by cork. Further increase in diameter is slight, and a cranberry stem always remains relatively slender, although it becomes very tough and woody as lignification of the internal tissues takes place. Pith, xylem, and rays, all become very thick-walled and lignified as shown by their reac-

tion with phloroglucin. The walls are distinctly pitted—pith and ray cells with simple pits, vessels and tracheids with pits of various types. Throughout the summer, cells of the pith, and to some extent those of the rays, were observed to contain large quantities of starch but very little oil.

The leaves remain functional for more than one year, and as they age become somewhat tough and leathery because of the heavy cuticle and the large amount of mechanical tissue associated with the main veins (plate XI, fig. 6). The mesophyll is surprisingly loose, with large intercellular spaces. During the summer both palisade and spongy cells usually contain large quantities of starch, as well as one or two globules of oil per cell. Oil is found in considerable quantity in the epidermis also. Calcium oxalate is conspicuous in terminal and axillary buds; in a mature leaf it is usually localized in the petiole and in the parenchymatous sheath that surrounds the veins. Stomata are confined almost entirely to the lower epidermis.

The infection of shoot tissues of ericaceous plants by the mycorrhizal fungus of these plants was first described by RAYNER (**9**) for *Calluna vulgaris,* and the discoveries have since been extended by her and several other workers to other plants, until such association of green plant and fungus has come to be regarded as a common condition in Ericaceae, although the details have been worked out for comparatively few plants.

A recent paper by RAYNER (**11**) describes in detail the distribution of the mycelium in the seed. The present authors have observed it in the ovary wall (plate XII, fig. 11) and in cells of the seed coat, but her more extensive researches have shown that it is present also in the endosperm of the resting seed. In the early stages of germination the tissues of the embryo become infected, and thereafter the infection of shoot tissues is assured. As cranberries are usually propagated by cuttings, mycelium is present in the stems when they are set out.

Study of the endophytic fungus in the shoot tissues is rendered difficult by its extremely small size, and by the fact that it is easily broken and washed away. Moreover, not all parts of all shoots are infected, so that prolonged and careful examination under high magnification may be necessary in order to detect the fungus in its relation to the cranberry plant. In general, fresh sections either unstained or stained lightly with mercurochrome or with ruthenium red, proved superior to microtome sections. Young mycelium was found to stain readily with ruthenium red and with aniline blue, indicating a content of pectic substances and of callose respectively; older mycelium did not take these stains.

In the present experiments, plants from all the various culture series were examined for the presence of the endophytic fungus. It was found in all, but least abundantly in the minus-nitrogen plants (Series 3). In

August the mycelium was usually most easily observed near the tips of rapidly growing runners. It was noted in practically all the different types of cells of the young stem except epidermal cells, but was most conspicuous in parenchymatous cells, especially of the pith (plate XII, fig. 9) and cortex. Usually some of the hyphae appeared to be in close association with the walls, but others extended into the protoplast. In cells that contained much mycelium, little or no starch was present and no chlorophyll. No starch grains were observed in the mycelium, but granules of glycogen were present, and at times globules of oil. Upon the addition of alcohol, crystals that were apparently glycine were also noted in the mycelium. Regardless of the concentration of nitrates in the external solution, no nitrates could be detected at any time in either the cranberry plant or its endophytic fungus.

In April and May the brown-walled mycelium in the older part of the stem was much more easily observed than the colorless mycelium near the rapidly growing tips, but as the season advanced it became less and less conspicuous because of the partial or complete disintegration, as shown in plate XII, fig. 12. Such disintegration or "digestion" has been described and figured by RAYNER (9) for *Calluna*. In old cranberry stems from both cultures and bogs the writers frequently observed dark masses of high stainability, especially in the conductive elements of the xylem and in the rays. In all probability they were phases of digestion of the fungus, for at no time could the writers detect the presence of other fungi such as those that are associated with the various diseases of cranberries. Dark masses, apparently similar to those just mentioned, were noted in leaves also, where they could be observed in greater detail. In leaves, however, they were in the mechanical tissue associated with the larger veins, rather than in the conductive elements of the xylem. Plate XI, fig. 6 and plate XII, fig. 10 show them as they appeared in cross and longitudinal section respectively. The smaller cells seemed to be entirely filled, whereas in the larger cells the masses were more open and apparently vacuolate. During the latter part of the season these dark masses were the only evidence of fungous invasion that could be noted in the leaves.

Discussion

A cranberry plant is thus in intimate association with an endophytic fungus. There are three possible methods of infection: (1) infection of young roots from external mycelium present in the soil, (2) infection of seedlings from tissues of the seeds at the time of germination, and (3) infection of plants propagated by cuttings from mycelium in the tissues of the cutting.

The nutritional physiology of such a complex offers many problems. It is said (**2, 8**) that *Phoma radicis* is capable of nitrogen-fixation, but the amount is small, and in these experiments proved entirely inadequate to support vigorous growth of the host, as shown by the poor growth of the plants of the minus-nitrogen series (Series 3).

In their natural state cranberries and many other ericaceous plants usually grow in soils that are conspicuously low in nitrates. In contrast to the general belief, the authors found by chemical test that a sample of bog soil may retain nitrates for several months after the addition of nitrate fertilizer. The fact remains, however, that under ordinary growing conditions the available supply of nitrates is very low.

Analysis of fig. 2 indicates that within the nitrate series (Series 1), smallest amounts of vegetative growth were associated with highest concentrations of calcium nitrate. Presumably the high concentration of the nitrate ion was a limiting factor. It is possible that the high concentration of calcium was toxic, but this seems unlikely, in view of the fact that although the minus-nitrogen (Series 3) plants received a relatively high concentration of calcium, they grew luxuriantly as soon as nitrogen in the form of ammonium sulphate was added. It also seems unlikely, from examination of fig. 2, that the poor growth of high-nitrate plants was caused by inadequate concentrations of either of the other two salts.

Although an excess of nitrate was directly or indirectly injurious, smaller amounts promoted growth. Nearly all of the plants of Series 1 exceeded in amount of runner growth similar plants that had grown for the same period in a bog. Moreover, when minus-nitrogen plants were supplied with a nutrient solution containing nitrates they showed appreciable increase in vegetative vigor, although recovery was slow. In these cultures ammonia was not present in the sand in quantities sufficient for detection by macrochemical methods.

As stated above, nitrates could not be detected at any time in either the cranberry plant or its endophytic fungus. An attempt was made therefore to determine whether at low temperatures nitrates might be absorbed but not assimilated. Such a condition is known to exist for asparagus (**7**). Cranberry plants were kept at temperatures of 2° C., 5° C. and 10° C. respectively for periods ranging from 8 to 48 hours. At no time could nitrates or nitrites be detected microchemically. When the plants were returned to higher temperature they resumed vigorous growth and were apparently uninjured by the treatment. It would thus seem that the absence of nitrates in cranberry plants is not associated with their rapid assimilation within the plant. In a consideration of this problem, the results of Dr. ECKERSON's experiments on reducase activity are significant.

Throughout the period of active growth, she made tests of various parts of plants, from both sand cultures and bogs. The extracted juice of even the finest roots contained only faint traces of material capable of reducing nitrates to nitrites. In a sample of older plants that were heavily infected with the endophyte, a trace of reducase activity could be detected. Results to date are too fragmentary to justify any conclusions as to the changes that take place between the initial absorption of nitrates, if such occurs, and the subsequent growth of new tissues.

It is significant that in amount of vegetative growth, plants of the ammonium series (Series 4) far exceeded those of the two nitrate series (Series 1 and 2), although they were started four weeks later. The intake of ammonium and its rapid passage through the plant were followed by means of Nessler's reagent and by the formation of crystals of ammonium chloroplatinate. Results of the present experiments suggest the ammonium sulphate in itself is not toxic to cranberry plants, although several field trials in New Jersey (1, 12) have indicated that under certain conditions it may not be used successfully as a fertilizer in cranberry bogs. Before definite conclusions can be reached, further experiments should be conducted with several different ammonium salts in several different concentrations, in cultures of several different degrees of acidity, and the results of these experiments should be correlated with those of further field experiments.

In the experiments described on page 657 it was found that dried blood, a commercial fertilizer that has a high content of organic nitrogen, stimulated practically as much vegetative growth as did ammonium sulphate in the concentration employed. Although it is impossible to state what changes took place between the time that organic nitrogen was applied to the culture and the time that nitrogenous compounds were absorbed by the plant or the mycorrhiza, it is interesting to note that analysis of sand from these dried blood cultures showed the presence of appreciable amounts of ammonium and amino nitrogen, but only traces of nitrates. It should also be noted that ammonia was found to be present in the sand of the cultures to which amino acids had been added.

It would thus seem from these preliminary experiments that ammonium sulphate can be used to promote vegetative growth of cranberry plants. Further experiments may show that the growth responses are modified by variations in acidity and other environmental factors. To what extent the green plant is aided by the endophytic fungus in the absorption and utilization of nitrogen has not yet been determined.

Summary

1. Cranberry plants were grown for several months in sand cultures supplied with nutrient solutions containing nitrogen in the form of nitrate

and ammonium respectively, and with a nutrient solution lacking in all forms of nitrogen. All except the minus-nitrogen series produced runner-growth exceeding that of similar plants grown in a bog.

2. Plants of the ammonium series produced noticeably greater runner-growth than those of the nitrate series.

3. Low concentrations of nitrate promoted vegetative growth, but high concentrations produced little growth under the conditions of these experiments.

4. Mycorrhiza was found in the cultures of all series, but least in the minus-nitrogen series.

5. The small amount of vegetative growth in the minus-nitrogen series indicated that if nitrogen-fixation by the endophyte (*Phoma radicis*) occurred, it was quite inadequate as a source of nitrogen for the cranberry plant.

6. Mycelium of the endophyte was found throughout the stem system of the plant, including fruits and seeds.

7. The mycelium forms a branching mass over the surface of the cranberry roots, which are very small and lack root hairs. Hyphae penetrate the epidermis and cortical parenchyma, and form mycelium in the cells.

8. In the stem, mycelium is most abundant in parenchymatous cells, especially of pith and cortex. It may be confined chiefly to the walls or it may be found throughout the protoplast. Oil and glycogen are often present in the fungus.

9. Nitrates could not be detected in either cranberry plant or endophyte at any time.

10. Experiments with organic nitrogen strengthen the conclusion that ammonium nitrogen can be utilized by the cranberry plant. The extent to which the endophytic fungus is concerned in the nitrogen-metabolism of the cranberry plant has not yet been determined.

The authors wish to acknowledge with sincere gratitude the counsel and active help of Dr. G. T. NIGHTINGALE throughout the progress of the experiments.

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LITERATURE CITED

1. BECKWITH, C. S. Effect of fertilizers on cranberry land. New Jersey Agr. Exp. Sta. Bull. 501. 1930.
2. DUGGAR, B. M., and DAVIS, A. R. Studies in the physiology of the fungi. I. Nitrogen-fixation. Ann. Missouri Bot. Garden **3**: 413–437. 1916.

3. ECKERSON, S. H. Microchemistry (mimeographed).
4. JANSE, J. M. Les endophytes radicaux de quelques plantes Javanaises. Ann. Jard. Bot. Buitenzorg **14**: 53–201. 1897.
5. JONES, L. H., and SHIVE, J. W. Effect of ammonium sulphate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulphate as sources of iron. Jour. Agr. Res. **21**: 701–728. 1921.
6. National Research Council, Div. of Biol. and Agr. A plan for coöperative research on the salt requirements of representative agricultural plants. Edited by B. E. LIVINGSTON. Second edition, Baltimore, 1919.
7. NIGHTINGALE, G. T., and SCHERMERHORN, L. G. Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.
8. RAYNER, M. C. Nitrogen-fixation in Ericaceae. Bot. Gaz. **73**: 226–235. 1922.
9. ————. The nutrition of mycorrhiza plants: *Calluna vulgaris*. Brit. Jour. Exp. Biol. **2**: 265–292. 1925.
10. ————. Mycorrhiza: an account of non-pathogenic infection by fungi in vascular plants and Bryophytes. New Phyt. Reprint no. 15. London, Wheldon and Wesley, 1927.
11. ————. The biology of fungus infection in the genus *Vaccinium*. Ann. Bot. **43**: 55–70. 1929.
12. SCHLATTER, F. P. Report on fertilizer experiments with cranberries. Proc. Amer. Cranberry Growers' Assoc. **48**: 9–12. 1917.
13. SHIVE, J. W., and STAHL, A. L. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. **84**: 317–323. 1927.
14. TERNETZ, C. Über die Assimilation des atmosphärischen Stickstoffe durch Pilze. Jahrb. wiss. Bot. **44**: 353–408. 1907.
15. VOORHEES, J. H. Experiments with fertilizers on cranberries. New Jersey Agr. Exp. Sta. Ann. Rept. 1914, pp. 247–251.

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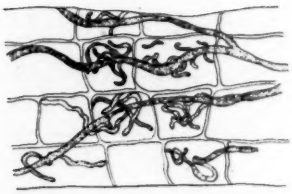

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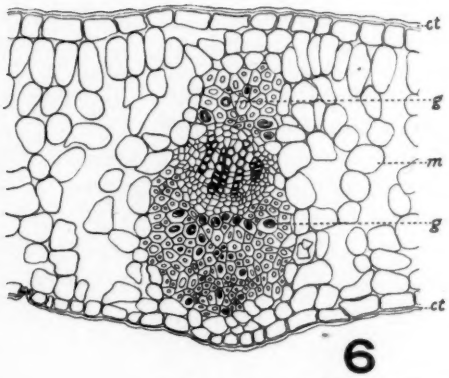

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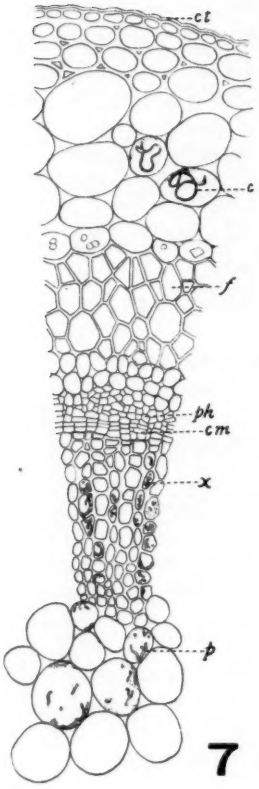

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ADDOMS AND MOUNCE: NOTES ON CRANBERRY

EXPLANATION OF PLATES

All drawings were made with the aid of projection apparatus, from fresh material except as otherwise noted.

PLATE XI

FIG. 4. External view of portion of root, showing mycelium on the surface. ×106.

FIG. 5. Portion of root, showing mycelium. Large hyphae are on the surface of the root, small ones inside the epidermal cells. ×480.

FIG. 6. Cross section of portion of leaf.
ct = cuticle
g = granular material in cells of mechanical tissue
m = spongy mesophyll
This drawing was made from a prepared slide. ×180.

FIG. 7. Cross-section of portion of young stem. ×180.
ct = cuticle
c = mycelium in cell of cortical parenchyma
f = fiber
ph = phloem
cm = cambium
x = mycelium in xylem
p = mycelium in cell of pith

PLATE XII

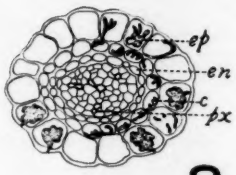
FIG. 8. Cross-section of young root.
ep = mycelium in epidermal cell
en = endodermis
c = mycelium in cell of cortical parenchyma
px = protoxylem point
This drawing was made from a prepared slide. ×180.

FIG. 9. Mycelium in cells of the pith of a young stem. ×480.

FIG. 10. Cells of the mechanical tissue of a vein of a leaf, in longitudinal section.
g = granular material

FIG. 11. Mycelium in cells of the ovary wall. ×480.

FIG. 12. Cells from the pith of a stem, showing stages in disintegration of the mycelium. ×480.



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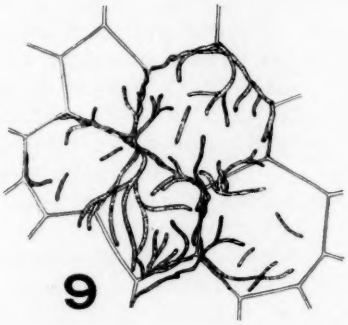

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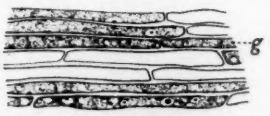

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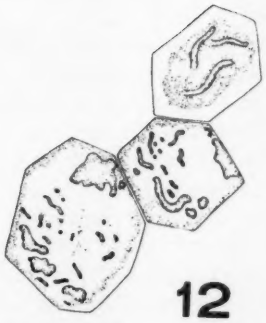

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ADDOMS AND MOUNCE: NOTES ON CRANBERRY

EFFECT OF CUTTING AND FERTILIZER APPLICATIONS ON GRASS DEVELOPMENT[1]

CARTER M. HARRISON

(WITH THREE FIGURES)

Introduction

The production and maintenance of turf on golf courses must take into consideration the varied uses to which the several parts of such a course are put. The ground cover of the "putting greens," "fairways," and "rough" differs markedly. The turf of the putting greens should be of such a density, height, and quality that a golf ball will roll evenly and smoothly over its surface. These characteristics, furthermore, must be maintained throughout the entire playing season even though the blades may be clipped daily to approximately three eighths or even three sixteenths of an inch in length. The fairway turf should consist of grasses whose blades stand firmly and stiffly upright so that they will hold a golf ball up on their ends, "teeing" it up for the next shot. The fairway is usually bordered by the rough. As the name implies, the grasses which compose it are permitted to grow longer; often those of a bushy or tufted nature are included.

Much difference of opinion prevails as to the best treatment of turf to secure a desired result. Factors in the environment coupled with the treatment to which the plants are subjected not only determine the kind or kinds of plants that can be grown, but also influences the growth produced by them during any given season.

Precise methods for the production and maintenance of desirable turf on the fairways of the modern golf course have not been developed. Short cutting of the grass on the fairways during the playing season is generally practiced. At times, it is cut to approximately one half inch. The closer the grass is clipped, the longer will be the intervals between the time of cutting and the time when the grass is long enough to prove troublesome to the game. Lengthening the intervals between cuttings results in a material saving in operative costs. In most instances some practice of fertilizing in addition to the cutting of the grass is necessary. These practices may in extreme instances be quite as harmful as beneficial to the growth of the grass. In fact it is easily possible to weaken or even kill grass by certain treatments, so that the ground is either left devoid of plant life or becomes covered with undesirable plants.

[1] Contribution from the Hull Botanical Laboratory, the University of Chicago, under a fellowship granted by the United States Golf Association Greens Section.

Much difficulty is encountered from the prevalence of low growing weeds, clover, and coarse summer grasses under close cutting treatments given the fairways, whereas the rough which is generally left uncut for longer periods of time or cut higher, is relatively free from such troubles. Also the rough usually stays green and thick after the fairways have become dry and sparse.

It would seem probable, therefore, that if the fairways could be cut in such a manner that a greater amount of leaf surface could be left than is true with the usual close cutting treatment, they too would remain in better condition. This result might be accomplished either by cutting the fairways close and less frequently, or by cutting frequently with the mower raised. The first method might prove troublesome because of the length which the grass would attain during intervals between cuttings, whereas the second would be feasible since it would provide a suitable playing surface and yet prevent serious injury to the grass because of too close clipping.

Some of the obvious detailed problems connected with the maintenance of grass turf are, (1) What response, relative to the quantity and quality of new growth, will grass plants exhibit after being cut at different heights? (2) Do the different species and varieties of grass respond similarly to any given treatment with respect to both the top and root growth produced? (3) What effect will cutting have upon the amount and length of root systems produced? (4) How do the treatments affect the ability of the plants to extend themselves vegetatively? (5) What are the effects of various fertilizer treatments on the amount of top growth produced by the plant in relation to the amount of such growth removed or allowed to remain?

In this investigation, solutions to certain of these problems were sought. The particular studies and experiments undertaken had to do with (a) the effect of frequent cutting to three different heights, one-half inch, one and one-half inches, and three inches upon the development and the dry weight of the roots produced; (b) the effect of cutting combined with fertilizing upon the length of roots produced; (c) the external structural responses of the grass to cutting and fertilizing as they relate to the quantity and quality of the turf; and (d) any anatomical variations which might be brought about by cutting, or fertilizing, or a combination of both.

Although considerable work has been done on agronomic herbaceous plants with respect to frequency and time of cutting, and with respect to the effect of fertilizer applications on the yield and permanence of stand, there are virtually no detailed records of experiments dealing with fairway maintenance.

As early as 1879 LAWES and GILBERT (**15**) found that various fertilizers greatly affected the relative numbers of different species of herbaceous plants present in a permanent meadow. Nitrogenous fertilizers, in general, favored the growth of grasses and reduced all other plants to a minimum, while the absence of nitrogenous fertilizers enhanced the growth of leguminous and other miscellaneous species. They also found that when nitrogen alone was used, the yield was increased considerably the first ten years of the experiment but dropped almost to the level of the checks the second ten years. KOSINSKI (**12**) and GODLEWSKI (**6**) showed that the addition of nitrogen salts to plants restricted the growth of roots in length and favored the growth of the stems. GODLEWSKI further showed that presence of sugar in a nitrogen free solution favored the growth of roots. HARRIS (**11**) found that wheat grown to maturity without fertilizer had a greater proportion of roots to tops, but where a nitrogenous fertilizer was added top growth was greater in proportion to the roots though the total root growth was greater in the latter case. GREEN (**9**) noted that in general frequent cuttings induced quick growth. KRAUS (**13**), ALBERT (**1**), and MCKEE (**16**) found that clipping of the tops of herbaceous plants retarded root development. SALMON *et al.* (**23**) found that cutting alfalfa at immature stages thinned the stand and lowered the yield. GREGORY (**10**) and CRIST and STOUT (**3**) stated that there appears to be a reciprocal effect between tops and roots of plants. WATERS (**29**), NELSON (**17**), and ALDOUS (**2**) showed that cutting frequently or cutting at immaturity depleted the reserves in the roots of herbaceous plants. NELSON also found a slow recovery after cutting, lower yields, and increased weed infestation, besides an increase on the plants of crown buds, shoots, and main stems, as a result of early and frequent cutting.

GRABER *et al.* (**7**), STURKIE (**28**), and PIERRE and BERTRAM (**19**) showed that the underground parts of alfalfa, Johnson grass, and kudzu could be reduced in weight below what they were at the beginning of the experiment by frequent and close clippings, and that the amount of reduction was associated with the severity of the cutting. PIERRE and BERTRAM found with kudzu that the roots of plants cut six times per season decreased in weight during a period of two years, those from plants receiving four cuttings increased about 150 per cent., those receiving two cuttings, about 400 per cent., and those receiving one cutting, about 1250 per cent. The percentage of reserve starch and nitrogen was found to be less than one-half as much in the roots of plants receiving six cuttings as in those of plants receiving four or fewer cuttings. The percentage of total sugars, however, was found to be greater. This was taken as an indication that a change from starch to sugar was taking place in the roots of plants receiving six cuttings, in order to produce new top growth. FITTS (**4**), working

with the fine turf grasses, found that the length of roots increased in relative proportion to the height to which the tops had grown. When the grass was surface fertilized, in order to produce a good turf, the roots were short, but when the grass was starved, the roots were longer and the turf was poorer. FAGAN, MILTON and PROVAN (5), in treating a mixture of grasses, grown from seed, with nitrate of soda, noted in the first harvest year that the response to the fertilizer was greater in the case of the grasses than of the clovers. This, coupled with the late growth of the clover, resulted in the latter, under most conditions of the investigation, being shaded and smothered by the grass. When the grass was cut at weekly intervals, the weight of clover in the total growth produced was equal to the weight of Italian rye grass (the most prominent grass). The yield of dry matter from the same area was much greater under a monthly system of cutting than under a weekly one. STAPLEDON and BEDDOWS (**25**), using orchard grass, concluded that repeated cuttings during the current season produced considerably less than the amount of a hay and aftermath crop. Repeated cutting reduced the root systems of the plants and retarded the growth produced early in the following spring. The pasture propagants gave as much as a 30 per cent. decrease in the amount of roots produced when compared with the hay plants and in some cases the difference amounted to 100 per cent. There was a wide response range to continued cutting in the pedigreed strains. Some resisted the treatment in a manner quite superior to that of others and showed only a slight reduction in roots. The plants with the highest yield did not necessarily have the heaviest root systems, but the nine highest yielding had roots somewhat heavier than the nine lowest yielding. Super-phosphate was without material quantitative or qualitative effects on root development, or on the stem to leaf ratio in the hay, aftermath, or pasture. Neither super-phosphate nor sodium nitrate alone was able to counteract the depressing influence of continued cutting.

REAM (**20**), STAPLEDON (**24**), STAPLEDON and MILTON (**27**) and STAPLEDON and DAVIES (**26**) found that root, tiller, or rhizome development and yield of the grasses studied were associated with the cutting treatment. The more drastic the cutting treatment, as measured by amount and frequency of defoliation, the less was the yield of roots, rhizomes and tops. The addition of nitrogenous fertilizers augmented the amount of yield of plant parts with a few exceptions where the cuttings were very short and frequent.

During the last fifteen or more years, several investigators have shown that chemical studies were pertinent to growth relations. KRAUS and KRAYBILL (**14**), NIGHTINGALE (**18**), and REID (**21**) have pointed out some apparent relationships between growth responses of plants and plant parts,

and the carbohydrate reserves and nitrogenous compounds within them. WELTON *et al.* (**30**) showed that the mowing of Canada thistles delayed organic food storage, resulting in a killing of the weaker plants. The more frequently the thistles were mowed, the more effective was the eradication. REID (**22**) showed that the relative amounts of carbohydrate to nitrogen compounds stored in seeds affected the ratio of tops to roots in the seedlings and that added nitrogen did little to augment growth unless light conditions were favorable for carbohydrate synthesis. GRABER (**8**) stated that frequent and close removals of the top growth of grasses having abundant reserves made for a heavy draft on the supplies of available nitrogen, so that the first limiting factor in growth might be nitrogen deficiency, but when regeneration was constantly stimulated by abundant mineral fertilizers, especially nitrogenous ones, the carbohydrate reserves were rapidly used with slight opportunity for replenishment and, as a result, they often became the principal factors limiting growth.

Experimental data

EXPERIMENT I

MATERIALS AND METHODS.—Preliminary greenhouse tests were made on three grasses in the spring of 1929. Three common golf course grasses were selected for the work, namely, Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), and Colonial bent (*Agrostis capillaris*). The flats in which the grasses were grown were twenty-two inches long, fifteen inches wide, and two and one-half inches deep. They were filled with a good grade of black top soil. The seed was sown at the following rates: Kentucky bluegrass, 1.91 grams per flat; Colonial bent, 1.43 grams per flat; and the red fescue, 2.86 grams. These rates correspond to 2.0 pounds, 1.5 pounds, and 3.0 pounds per thousand square feet respectively.

The seed was sown on March 13th, the experiment was set up in triplicate, and the cutting treatment was started April 15th. The grass was cut to three heights, one-fourth inch, one and one-half inches, and three inches. In addition three flats of each grass were clipped short and ammonium sulphate was added on April 30th and May 22nd. The first application was made at the rate of two pounds per thousand square feet and the second at the rate of seven pounds per thousand square feet. The grass in all of the flats was cut sixteen times between April 15th and July 5th.

Beginning July 5th, the grass roots were washed from the soil with as much care as possible. The roots were distinctly matted in the grass cut to the higher levels, but this was not true of the most closely clipped flats.

RESULTS.—The results are recorded in table I in grams per square foot of surface area.

TABLE I

WEIGHT OF ROOTS IN GRAMS PER SQUARE FOOT OF SURFACE AREA

VARIETY	RED FESCUE		BLUEGRASS		COLONIAL BENT	
TREATMENT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Short cut, ¼ inch	6.8	1.4	8.5	1.6	13.8	2.1
Short cut, ¼ inch and ammonium sulphate	4.3	1.0	6.8	1.3	Killed	
Medium cut, 1.5 inches	51.6	8.6	36.3	7.5	29.4	5.0
Long cut, 3 inches	78.0	13.7	61.0	11.7	59.1	7.7

Table I indicates that there is a considerable difference in the amount of roots produced as a result of the different cutting treatments and also by the different grasses. The root systems of the bent grass which was cut short weighed nearly double that of either of the other two grasses receiving the same treatment. The percentage increase in roots between plants when cut at three different heights is large and indicates a direct correlation between the amount of top growth and the amount of root growth produced.

The character of the response of the short clipped bent grass that was fertilized to the combination of a heavy nitrogenous fertilizer and shading of the greenhouse was interesting. The grass had been growing in a greenhouse that was not shaded. Until the greenhouse was covered to cut down the light, these flats were a beautiful green, while the flats having no nitrogen, and clipped short, were yellowish in color. No later than three days after the shade mixture had been applied to the greenhouse roof, the heavily fertilized grass rapidly took on a scalded appearance until only the plants near the sides of the flat showed a healthy green color. In the meantime, the grass that was clipped short and that had received no nitrogen turned from a yellowish color to a dark green. The plants which appeared scalded died and after examination of the material their death could not be attributed to fungus troubles. Appearing as it did about a week after a heavy fertilization with nitrogen and a decrease in the light intensity, it was thought that possibly in the absence of sufficient manufactured carbohydrates, the nitrogen was toxic and brought about the scalded condition.

EXPERIMENT II

GREENHOUSE EXPERIMENT WITH KENTUCKY BLUEGRASS AND RED FESCUE.—Because the flats used in the first experiment were too shallow to allow the

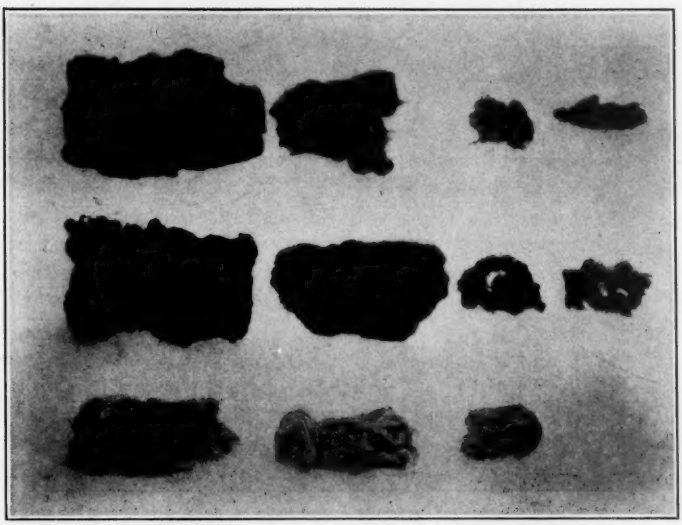

FIG. 1. Photograph showing bulk of roots produced by different grasses when cut to three heights and when nitrogen is added to short cut grass.
Upper row: Kentucky bluegrass. Reading left to right: cut long, cut medium, cut short, and cut short plus nitrogen.
Middle row: Red fescue. Treated same as bluegrass.
Lower row: Colonial bent. Treated same as bluegrass. Roots from the grass plants that were fertilized and clipped short are missing because the grass killed.

roots of the plants to grow downward and because they dried out too rapidly, another experiment was begun in the fall of 1929 to duplicate in part and supplement in some features the preliminary study.

MATERIALS AND METHODS.—Wooden boxes of approximately one cubic foot volume were obtained. These were filled with a mixture of eight parts of black soil, two parts of quartz sand, and one part of leaf mold. The soil was sifted to remove large particles of organic material and gravel. The seed was sown on September 25th.

Fertilizer applications were made once per month during January, February, and March. The applications consisted of the following:

1. Nitrogen, 1.12 grams of ammonium sulphate.
2. Nitrogen-phosphorus, 1.12 grams of ammonium phosphate.
3. Nitrogen-potassium, 0.85 grams of potassium nitrate plus 0.57 grams of ammonium sulphate.
4. Nitrogen-phosphorus-potassium, 1.12 grams of ammonium sulphate plus 1.15 grams of monobasic potassium phosphate. The amount of nitro-

TABLE II

DRY WEIGHT OF ROOTS IN GRAMS PER SQUARE FOOT FOR THE DIFFERENT CUTTINGS AND FERTILIZER TREATMENTS IN KENTUCKY BLUEGRASS AND RED FESCUE

		Bluegrass						Fescue					
		Cut ½ inch	± mean	Cut 1½ inch	± mean	Cut 3 inch	± mean	Cut ½ inch	± mean	Cut 1½ inch	± mean	Cut 3 inch	± mean
Check	1	2.6	−0.03	7.8	−0.07	10.7	−1.47	4.7	−0.23	7.5	−0.90	13.8	−0.63
	2	3.0	+0.37	8.3	+0.43	11.5	−0.67	4.5	−0.43	9.1	+0.70	16.1	+1.67
	3	2.3	−0.33	7.5	−0.37	14.3	+2.13	5.6	+0.67	8.6	+0.20	13.4	−1.03
Nitrogen alone	1	4.3	+0.47	6.8	−0.60	10.0	+0.57	7.0	+0.93	9.2	+0.43	11.0	−0.03
Ammonium sulphate	2	3.3	−0.53	7.4	± 0.0	9.5	+0.07	5.2	−0.87	8.9	+0.13	10.6	−0.43
	3	3.9	+0.07	8.0	+0.60	8.8	−0.63	6.0	−0.07	8.2	−0.57	11.5	+0.47
Nitrogen and phosphorus	1	3.2	+0.23	6.5	−0.50	8.9	−0.63	5.2	+0.23	8.5	+1.0	10.3	+0.60
Ammonium phosphate	2	2.4	−0.57	7.1	+0.10	10.6	+1.07	4.8	−0.17	6.8	−0.70	9.5	−0.20
	3	3.3	+0.33	7.4	+0.40	9.1	−0.43	4.9	−0.07	7.2	−0.30	9.3	−0.40
Nitrogen and potassium	1	4.0	+0.30	8.1	+0.17	8.5	+0.50	5.6	+0.20	7.0	+0.03	9.8	+0.03
Potassium nitrate and	2	3.9	+0.20	8.6	+0.67	8.3	+0.30	5.4	± 0.0	8.1	+1.07	10.5	+0.73
ammonium sulphate	3	3.2	−0.50	7.1	−0.83	7.2	−0.80	5.2	−0.20	6.0	−1.03	9.0	−0.77
Nitrogen phosphorus and potassium	1	3.1	+0.67	7.8	−0.03	10.0	+0.70	5.6	+0.07	7.1	+0.23	11.5	+0.43
Potassium acid	2	2.2	−0.23	8.4	+0.57	9.2	−0.10	5.9	+0.37	6.3	−0.57	10.5	−0.57
phosphate and ammonium sulphate	3	2.0[1]	−0.43	7.3[1]	−0.53	8.7[1]	−0.60	5.1[1]	−0.43	7.2[1]	+0.33	11.2[1]	+0.13

[1] Weight of roots of the flats from which the photographs were obtained.

gen added to the grass in each flat was the same regardless of the salt used. The experiment was set up in triplicate.

Beginning November 7th the plants were cut and the cutting continued each week thereafter until the close of the experiment. Fifteen flats of each species of grass were cut to one-half inch, fifteen to one and one-half inches, and fifteen to three inches. All clippings were removed from the flats. Beginning April 21st the soil was washed from the roots. The dry weight of the roots was recorded, the results being given in table II.

From table II it is obvious that the cutting heights made considerable difference in the amount of roots produced and that very little can be said in favor of the fertilizer treatments with respect to root growth. More difference is apparent between the triplicates of a certain treatment than between treatments.

Besides weighing the roots produced by the different treatments, it was thought advisable to select a flat of grass of each of the three cutting heights where the best top growth was obtained from which to get photographs of the height of cut, the density of the turf, and the length and amount of roots produced. The complete fertilizer gave what appeared to be the best top growth at all three heights. The fescue was selected likewise. The rhizomes were carefully picked out of the three fertilized bluegrass flats, and likewise out of three check flats, and the oven dry weight was recorded as in table III.

TABLE III

Treatments	Total dry weight of rhizomes per flat
	gm.
Bluegrass—clipped long, potassium phosphate and ammonium sulphate	0.726
Bluegrass—clipped long, check	0.594
Bluegrass—clipped medium, potassium phosphate and ammonium sulphate	0.400
Bluegrass—clipped medium, check	0.225
Bluegrass—clipped short, potassium phosphate and ammonium sulphate	0.018
Bluegrass—clipped short, check	0.008

Many more rhizomes were produced by the grass cut high than by that cut to a medium height, and decidedly more were produced by the grass cut to a medium height than by that cut short. In fact, very few of the plants in the short cut flats had visible rhizomes.

Sections were cut of representative plants selected from the checks and from those receiving nitrogen, in the three cutting heights. This was done on both the fescue and the bluegrass. Nothing could be noted from the

FIG. 2. Three bluegrass flats to which a complete fertilizer had been added, showing: above, the height of cut; middle, the turf produced at those heights; and below, the mass and length of roots produced.

slides that was not apparent in the external structure of the plants. It was observed that the shorter cutting of the fescue induced much more tillering than was the case in those cut longer. The grass cut to a medium

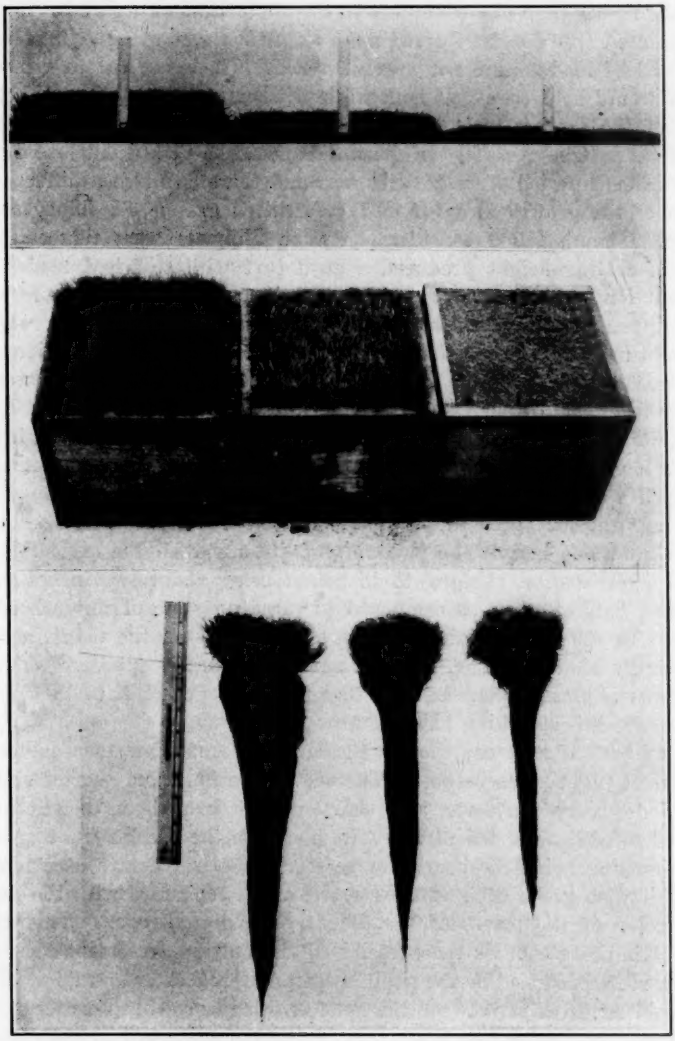

FIG. 3. Three fescue flats to which a complete fertilizer had been added, showing: above, the height of cut; middle, the turf produced at those heights; and below, the mass and length of roots produced.

height had stiffer and more upright leaf blades than that cut long, the long cut plants having a tendency to lop over. This was probably due to length

of blade rather than to a difference in the anatomical structures of the two. The plants clipped short tillered more than those cut to a medium height, but the leaf blades were not nearly so stiff. The grass cut to a medium height would hold up a golf ball, while neither of the others would do so. None of the cutting treatments appeared to result in a thinning of the fescue turf. The grass in the check flats could be picked out late in February after a period of bright weather, and there was also a difference in length of top growth in favor of the fertilized grass. The bluegrass, on the other hand, killed considerably when clipped short, that with the medium cutting height produced a good turf with stiff leaf blades, and that cut long produced a turf that lopped over as did the fescue which was clipped high. The effect of the nitrogen on the bluegrass was not apparent until late in February after the second fertilizer application. All the grass had been a dark green color until this time when the weather became bright and clear as contrasted with the cloudy, sunless period preceding it. During these sunny days, the checks in all three cutting heights turned a greenish yellow while the grass in the flats receiving nitrogen remained a dark green. Between cutting periods, the grass clipped long receiving nitrogen grew approximately three inches, while that in the checks grew one inch. The medium cut grass showed the same response but to a less degree, the growth in height being about two inches in the fertilized flats of grass as compared to three quarters of an inch in the checks. In the flats of grass cut short and fertilized, the plants sent up one spindly blade, the turf thinned out badly, and it appeared as if the nitrogen was harmful here because the grass in the check flats did not thin out or have this spindling habit of growth.

It was noted that the shorter the clipping treatment the smaller the subsequent plant parts became. The roots were finer and shorter and the leaf blades were narrower and shorter. The growth in height corresponded to the size of the plant. The plants in the check flats, regardless of the cutting height, appeared to be storing carbohydrates as evidenced by a yellowish green color of their leaves when compared with the darker green color of the leaves of the plants receiving nitrogen. This might possibly indicate that as the severity of the cutting treatment increased, the new roots produced by the plant became fewer, shorter and finer. Thus a gradual adjustment between the amount of top growth and root growth took place, the final result being that in no case was the root system sufficiently extensive to gather enough nitrogen to prevent carbohydrate storage.

Summing up the general observations, it appears as if the nitrogen had resulted in increased top growth in all cases except the short clipped bluegrass. The fescue which was cut short tillered more than that cut long. That cut at a medium height, however, had the most desirable leaf blades from a golf course view-point.

The foregoing data show that the shorter the grass was clipped, the fewer roots were produced. It has been shown by several workers that frequent removal of top growth influenced the subsequent yield of tops, lessened the carbohydrate storage, and if the clipping were severe enough, no carbohydrates were stored. It has also been noted that when the tops are severely clipped, the weight of the root systems may be reduced below the initial weight at the start of such treatment. Lessening the leaf area resulted in the production of fewer, smaller roots than was the case when the treatment was not so severe. The size of the successive leaves produced by the plant depended on the cutting treatment: the more severe the treatment, the smaller they became. As the clipping treatment became more and more severe, generally less and less leaf area was left to manufacture carbohydrates, which are necessary both for the growth of roots and for subsequent top growth.

Removal of photosynthetic surface can be carried to such an extreme that the grass plant is weakened to such a degree that it can no longer survive either drouth, disease, extremes of heat or cold, or the competition of plants which have growth characteristics enabling them to escape the close clipping by tillering, or by growing almost flat on the surface of the ground. These plants, such as white clover, dandelion, crab grass, knotweed, and plantain, retain sufficient leaf area to maintain themselves under clipping practices which are very detrimental to the growth of upright growing grasses such as Kentucky bluegrass. Bluegrass continues to grow erect, and the new chlorophyll bearing tissue is continually cut off as it is produced. Field observations as well as greenhouse studies show that Kentucky bluegrass cannot maintain itself under ordinary field conditions if it is cut shorter than three-quarters of an inch more frequently than once each week. The plants extend themselves very little vegetatively when these practices are followed. The rhizomes formed are few in number and grow very slowly. If the grass is allowed to grow longer or is cut less frequently, there is sufficient production of rhizomes to insure good perennial turf, and besides, the undesirable plants are kept hidden or are crowded out by the vigorous growth of the grass. Even among the different grasses, there are species that can maintain themselves where others are killed by the short and frequent clipping. Most of the vegetative and seeded bent grasses that are in use today for turf purposes produce considerable chlorophyll-bearing tissue near the surface of the ground in such a manner as to escape close clipping. It is this characteristic that makes them valuable for the putting greens. Red fescue tillers when cut short, and produces many leaves close to the ground away from the mower blades. Therefore it is able to withstand more severe cutting than grasses which do not have this habit of growth.

The relationship that exists between carbohydrates and nitrogen in plant nutrition has been pointed out by workers in the field, and it has been shown that an application of nitrogen is of little use in subsequent growth unless the plant has an available supply of carbohydrates or is capable of manufacturing it. An excess of mineral fertilizer cannot take the place of carbohydrates in plant growth. The appended data show in general that root growth will not respond to fertilizer applications if the ability of the plant to manufacture carbohydrates is impaired by short clipping. The mowers should be raised on the fairways where the cutting treatment is proving dangerous, and cutting should be discontinued in the fall as early as possible in order that the plants may store foods for use during the season of short and frequent clipping.

Summary

1. Clipping at different heights affected the amount of roots produced on the grasses studied. The shorter the grass was cut and the more the leaf area was reduced, the smaller was the quantity of roots produced.

2. Grasses having different growth characteristics responded differently to the cutting treatments. The fescue tillered more when cut short than when cut long, and the bluegrass produced fewer rhizomes when cut short than when cut long.

3. Mineral fertilizers did not compensate for a lack of top growth in the production of roots.

4. The killing of the grass was not due to a cutting off of the buds, but to a gradual carbohydrate starvation to a point beyond which the plants could not maintain themselves.

5. The addition of nitrogen brought about an increase in top growth, but the weight of roots was not increased over that of the roots of the unfertilized grass.

LITERATURE CITED

1. Albert, W. B. Studies on growth of alfalfa and some perennial grasses. Jour. Amer. Soc. Agron. **19**: 624–660. 1927.
2. Aldous, A. E. Relation of organic food reserves to the growth of some Kansas pasture plants. Jour. Amer. Soc. Agron., **22**: 385–392. 1930.
3. Crist, J. W., and Stout, G. J. Relation between top and root size in herbaceous plants. Plant Physiol. **4**: 63–85. 1929.
4. Fitts, O. B. Root growth of fine grasses. Greens Section Bull. U. S. G. A. **5**: 58–62. 1925.
5. Fagan, T. W., Milton, W., and Provan, A. The effect of nitrate of soda on the yield and chemical composition of a simple seeds

mixture in the first harvest year. Bull. Welsh Plant Breeding Sta. Aberystwyth. Series H., no. 9. 1928.

6. GODLEWSKI, E. Zur Kenntnis der Eiweissbildung in den Pflanzen. Bull. Internat. Acad. Sci. Cracovie. 313–380. June. 1903.
7. GRABER, L. F., NELSON, N. T., LUEKEL, W. A., and ALBERT, W. B. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wisconsin Agr. Exp. Sta. Res. Bull. 80. 1927.
8. ————. Food reserves in relation to other factors limiting the growth of grasses. Plant Physiol. **6**: 43–71. 1931.
9. GREEN, A. W. Grasses at Ruakura. Jour. New Zealand Dept. Agr. **5**: 216. 1912.
10. GREGORY, F. G. Effect of temperature and light on the increase of leaf surface. Ann. Bot. **42**: 469–507. 1928.
11. HARRIS, F. S. The effect of soil moisture, plant food, and age on the ratio of tops to roots in plants. Jour. Amer. Soc. Agron. **6**: 65–75. 1914.
12. KOSINSKI, G. Uplyw zwiazkow azotū na rozrost róslin Kielkujacych. Roczniki nauk rolniczych. Krakow. 1907.
13. KRAUS, C. Untersuchungen zu den biologischen Grundlagen des Grasbaues. Fühling's Landw. Zeitung 61, Jahrgang (10) and (11). 1911.
14. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
15. LAWES, J. B., and GILBERT, J. H. Agricultural, botanical, and chemical results of experiments on the mixed herbage of permanent meadow conducted for more than twenty years in succession on the same land. Phil. Trans. Roy. Soc. London **171**: 289–416. 1881, and **173**: 1181–1413. 1883.
16. MCKEE, ROWLAND. The effect of clipping on the root development of alfalfa. Jour. Amer. Soc. Agron. **8**: 329–332. 1916.
17. NELSON, N. T. The effects of frequent cutting on the production, root reserves, and behavior of alfalfa. Jour. Amer. Soc. Agron. **17**: 100–113. 1925.
18. NIGHTINGALE, G. T. The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. 74. 1927.
19. PIERRE, W. H., and BERTRAM, F. E. Kudzu production with special reference to the influence of frequency of cutting on yields and formation of root reserves. Jour. Amer. Soc. Agron. **21**: 1079–1101. 1929.

20. REAM, H. Some studies on root reserves in *Poa pratensis* and *Agropyron repens*. B.S. Thesis, Univ. of Wisconsin, June, 1930.
21. REID, M. E. Relation of kind of food reserves to regeneration in tomato plants. Bot. Gaz. **77**: 103–110. 1924.
22. ————. Growth of seedlings in light and in darkness in relation to available nitrogen and carbon. Bot. Gaz. **87**: 81–118. 1929.
23. SALMON, S. C., SWANSON, C. O., and MCCAMPBELL, C. W. Experiments relating to the time of cutting alfalfa. Kansas Agr. Exp. Sta. Techn. Bull. 15. 1925.
24. STAPLEDON, R. G. The seasonal productivity of herbage grasses, with particular reference to the influence of different systems of cutting on indigenous and non-indigenous strains, respectively. Bull. Welsh Plant Breeding Sta. Aberystwyth. Series H., no. 3. 61–70. 1924.
25. ————, and BEDDOWS, A. R. The quantitative and qualitative response of cocksfoot (*Dactylis glomerata* L.) to sodium nitrate and to superphosphate. Welsh Jour. Agr. **2**: 103–113. 1926.
26. ————, and DAVIES, WM. Experiments to test the yields and other properties of various species and strains of herbage plants under different methods of management. Bull. Welsh Plant Breeding Sta. Aberystwyth. Series H., no. 10. 1930.
27. ————, and MILTON, W. The effect of different cutting and manurial treatments on the tiller and root development of cocksfoot. Welsh Jour. Agr. **6**: 166–174. 1930.
28. STURKIE, D. G. The influence of various top-cutting treatments on rootstocks of Johnson grass (*Sorghum halepense*). Jour. Amer. Soc. Agron. **22**: 82–93. 1930.
29. WATERS, H. J. Studies of the timothy plant. I. The influence of maturity upon the yield, composition, digestibility, palatability, and feeding value of timothy hay. Missouri Agr. Exp. Sta. Res. Bull. 19. 1915.
30. WELTON, F. A., MORRIS, V. H., and HARTZLER, A. J. Organic food reserves in relation to the eradication of Canada thistles. Ohio Agr. Exp. Sta. Bull. 441. 1929.

EFFECT OF X-IRRADIATION UPON GROWTH AND REPRODUCTION OF TOMATO*

EDNA LOUISE JOHNSON

(WITH THREE FIGURES)

Experimentation (**2**) has shown that plants differ in the way that they react to X-ray exposure. The tomato, *Lycopersicum esculentum,* is less affected by the action of these rays than are some other species. Since in a preliminary study seeds given a medium dose produced plants which appeared normal in all respects, all later irradiation was made directly on the seedling.

Materials and methods

Seven different sets of experiments (series I–VII) were carried on, in most of which the John Baer variety of tomato was used. In the first three series irradiation was carried out when the plants were from 1 to 3 centimeters in height; in the fourth, three medium doses were given at two-week intervals,—at a time when the plants seemed to be recovering from the effects of the previous dose. In the last three groups, the plants were not exposed until small buds had formed.

The general procedure was to plant seeds in 5-inch pots in the greenhouse. The plants of the first three series were irradiated before the true foliage leaves had developed. Soon afterwards these seedlings and an equal number of controls were transplanted to larger pots where they grew to maturity. Plants of series V, VI, and VII were transplanted at an early age but were not irradiated until small buds had formed.

Irradiations of the first five groups were made with a Victor X-ray machine with the following "set-up": 5-inch spark gap, 5 milliamperes current, no filter, distance 30 cm., time from 20 to 30 minutes. For the last group, an Acme machine was used with the potential 90 K.V., 5 milliamperes current, no filter, distance 30 cm., time 20 minutes.

Results

EFFECT ON THE LEAVES OF NEW GROWTH

The first noticeable effect on the plant is a peculiar warty or pebbly appearance of the young foliage leaves which is evident soon after irradiation. As the leaves grow older, they show light green areas intermingled with the ordinary green thus giving a mosaic or variegated aspect. Microscopic

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examination of the leaves indicated that there had been an interference with the normal development of palisade and spongy parenchyma. The walls of the palisade cells particularly seemed to lack distinctness of outline. The chloroplastids appeared contracted and more blue-green in color than did the plastids of the controls.

The cotyledons remained entirely normal, but marked leaf abnormalities were evident in young tissue developing after irradiation. In many cases, for half the length of the leaflet, the blade on one side was absent. Leaflets were often twisted for one-half or one-third of their length. Fusion of leaf parts was common. Often the widened base of the leaflet grew to the main rachis so that no petiole at all was present. One case was noted where a lateral leaflet showed the tip edges attached together to form a cup-like structure. The edges of the leaflets were sometimes grown to the main rachis or with adjacent leaflets. In some cases marked leaf anomalies were not evident, but one could always distinguish irradiated plants by the pebbly appearance of the young leaves.

Changes in growth form

A change in the general aspect of the entire plant, resulting from greater development of lateral branches is another condition noticeable in every group of tomato plants given medium dosage. A special study of this trait was made in series IV, V, and VI. In series IV, the dose was repeated twice at approximately two-week intervals. The last dosage was given when the buds had formed. Earlier experiments (2) had established the fact that immediately after irradiation with a dose of medium strength, a growth depression occurs which later tends to disappear. Those employing X-rays for therapeutic purposes find that a similar condition holds true for a treatment of human diseases. The dose is repeated between the second and third week, as the effects of the treatment usually disappear by the end of the third week. It seemed desirable to compare effects produced by giving doses to plants at various intervals with the results produced by a single exposure at the budded stage. Series IV was given three medium doses at intervals while the plants in series V, VI, and VII received but one each at the time small buds had formed.

The bushy appearance presented by the irradiated plants is due to the greater development of lateral branches. A comparison of this feature in the irradiated and control plants is shown in table I. All lateral branches one centimeter or more in length were counted and measured. In series IV, there was an increase in branch development of 27.5 per cent. over that found in the control plants. In series V and VI where but one exposure was given to plants which had already formed buds, the percentage of increase of total length of branches from irradiated plants over the control

TABLE I

LATERAL BRANCH DEVELOPMENT

	Series IV			Series V			Series VI		
	Control	Irradiated[1]	Increase of irradiated over control	Control	Irradiated[2]	Increase of irradiated over control	Control	Irradiated[3]	Increase of irradiated over control
			per cent.			*per cent.*			*per cent.*
Days after radiation when measurements were made		47.0			35.0			50.0	
Age when radiated		17.0			62.0			55.0	
No. of plants	11.0	11.0		12.0	12.0		11.0	11.0	
Total no. of side branches	76.0	78.0	2.6	10.0	22.0	120.0	20.0	28.0	40.0
Av. no. per plant	6.9	7.1	2.8	0.75	1.83	140.0	1.81	2.54	40.0
Total length (cm.)	410.0	522.8	27.5	100.0	156.4	56.4	66.0	109.0	65.1
Average length (cm.)	5.39	6.70	24.3	10.0	7.1	−29.0	3.35	3.9	16.4

[1] Irradiated with 3 medium doses (5 in. spark, 5 milliamp., 30 cm. for 20 min.) at intervals of approximately two weeks.

[2] Irradiated with one dose (5 in. spark, 5 milliamp., 30 cm. for 20 min.)

[3] Irradiated with one dose (90 K.V., 5 milliamp., 30 cm. for 20 min.)

was considerably greater than in Series IV, whereas the average length was less.

Results in table I indicate that tomato plants irradiated either in the seedling stage or at the time that buds are appearing produce a greater number of lateral branches per plant than controls of the same age. In two of the three groups, the average length of branches from the irradiated plants was also greater.

Effect on floral development

Observations were made on four series of plants to determine the effects of irradiation on the time of blossoming and the occurrence of anomalies in buds and blossoms. The normal tomato flower is perfect, regular, and typically six-merous. The calyx tube is very short, and bears six leafy lobes which are linear to lanceolate. The six or more yellow stamens are laterally joined to form a hollow cone around the pistil. The pistil has an elongated style, and the stigma extends slightly beyond the apex of the androecium. Many types of anomalies were found in blossoms developed from buds which had been X-rayed. Table II gives a list of abnormalities present in blossoms of series IV, seventeen days after the last exposure. In this group, three exposures were made, the first on 17-day old seedlings, the last when buds were barely visible. Only those blossoms which were fully open at the time of observation, or those which had just faded, were included in the list.

TABLE II

Anomalies found in blossoms of irradiated plants of Series IV*

Description of anomaly	Number of cases
Double blossom	2
Corolla showing more than 6 lobes	16
Lobes of corolla cleft	3
Corolla lobes badly twisted	3
Calyx with more than 6 lobes	4
Lobes of calyx cleft	2
Fusion of calyx lobes	13
Twisted stamens	12
Total	55

* At the time of observation, there were 24 blossoms on the 11 irradiated plants under observation.

On the same day that the above observations were made on the irradiated plants, there were 16 blossoms present on the 11 control plants. Fifteen of these had six corolla lobes and six calyx lobes.

In view of the results given above, it seemed probable that similar anomalies could be produced by allowing plants to come to the budding stage and then irradiating them with one medium dose. With the last three series, plants were irradiated when they were approximately 60 days old. With some plants, the only part of the bud which showed any growth after irradiation was the pistil. Frequently the style became greatly elongated protruding from the calyx tube. Often the style showed marked flattening. In many cases, the buds present at the time of irradiation became yellow and dropped off. In one case, the irradiation affected the plant so that buds did not develop. Table III summarizes the floral development of the last three series.

TABLE III

DEVELOPMENT OF FLOWERS FROM FIRST BUD CLUSTER

	Series V. 62-day old plants irradiated with one dose from following set-up: 5 inch spark gap, 5 milliamp., 30 cm. for 20 min.		Series VI. 60-day old plants irridiated with one dose from following set-up: 90 K.V., 5 milliamp., 30 cm. for 20 min.		Series VII. 55-day old plants irradiated with one dose from following set-up: 90 K.V., 5 milliamp., 30 cm. for 20 min.	
	Control	Irradiated	Control	Irradiated	Control	Irradiated*
No. of plants	12	12	11	11	7	7
Total no. of blossoms	56	1	43	13	25	23
Average no. of blossoms	4.66	.083	3.9	1.18	3.57	3.28
Ave. no. of days for blossoms to appear	17.4	.....	24.3	20	25.4	29.3
Percentage of plants never developing flowers in first bud-cluster	0	91.6	0	63.6	0	0

* Lessened effect probably due to fact that plants were irradiated when buds were younger than those in the other series. Leaf anomalies were as evident as in other irradiated plants.

A comparison of flower development in series IV and V indicates an existence of radiophylaxis which ANCEL and LALLEMAND (1) have demon-

strated. In their experiments, it was found that an exposure to a light dose preceding the heavy dose lessened the effect of the heavier dose which followed. In series IV one medium dose which was given to the 17-day old seedlings was repeated at two-week intervals, the last being given when small buds had formed. When these plants were 3.5 months old, 9 per cent. of the irradiated plants, and 63.6 per cent. of the controls, were bearing fruit. When these same plants were 5 months old, 54.5 per cent. of the irradiated, and 90.9 per cent. of the controls, were fruiting. With series V where the plants were exposed to but one medium dose, irradiated plants 3 months old had produced no fruit while 75 per cent. of the controls were bearing fruit. When these plants were 4 months old, 25 per cent. of the irradiated, and 92 per cent. of the controls, were in fruit.

The plants which had two previous exposures before irradiation did not drop their buds but developed flowers. These showed many malformations of structures, it is true, but the injury was considerably less than that sustained by the plants of series V in which only one bud that received irradiation ever developed a flower. The X-rays apparently hastened the development of the abscission layer of the pedicel for, soon after the exposure, the region of the pedicel where the abscission layer develops became yellow and the bud soon dropped. This case seems to illustrate the phenomenon of radiophylaxis, as the earlier doses given the plants apparently prevented the serious injury which results when one dose only is given at the time the buds are formed.

An accurate record of the time of blossoming and the number of blossoms appearing in the second bud cluster was kept for series VI and VII. In both of the series there was but little difference in the time of blossoming between the experimental plants and controls. The average number of blossoms, however, was greater in the irradiated plants. This will be noted particularly in future study in order to determine whether inhibition of flower and fruit development from the first bud cluster will be accompanied by increased vegetative and reproductive growth of the irradiated plants. In series IV and V double and triple flowers were produced. Figure 1 shows two of the unusual flowers produced. One was a double structure as is shown by the presence of two pistils with the surrounding androecia. The second flower was a triple one with three separate pistils each surrounded by a complete androecium.

Fruit development

All groups of tomato plants were allowed to grow to maturity so that fruit development could be observed. In series I, buds appeared on irradiated plants at about the same time as on the controls. Fruit development seemed in no way affected by irradiation. In the plants of series II where

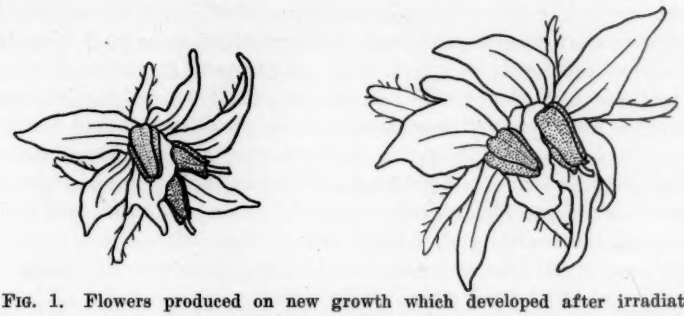

FIG. 1. Flowers produced on new growth which developed after irradiation of plants with a medium dose. At left, triple flower showing three pistils with their surrounding stamens. In two of the flowers, the stamens are twisted so that a portion of the style is exposed. At right, double flower with two pistils and their surrounding stamens.

a slightly heavier dose was given, fruits appeared on the irradiated plants at a somewhat later date than on the controls. When a still heavier dose was given to plants of series III, fruit development was still more retarded.

In plants of series IV where a medium dose was given three times, fruit production was slow for a time. Later, when the plants had time to recover from the last dose, the number of fruits more nearly equalled those

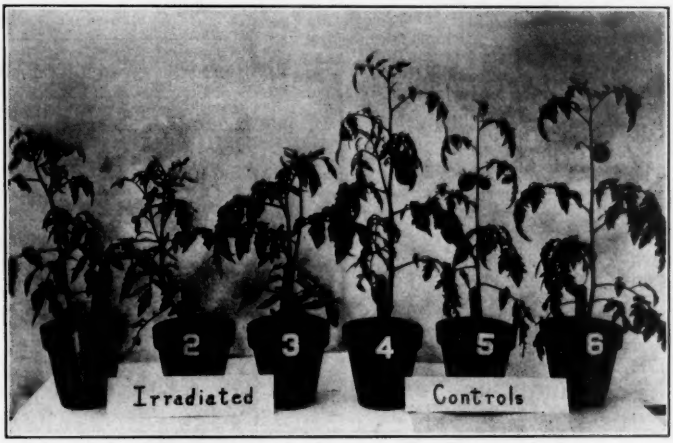

FIG. 2. Two-month-old tomato plants irradiated when the buds were very small presented this appearance 5 weeks later. Note the lessened height, branching habit, and sterile condition of the experimental plants. Buds have not developed on the deformed, abnormal growth. Controls show normal growth form and well developed fruit with new buds forming.

of the controls. In series V which was composed of a group of plants which were not irradiated until small buds were produced, none of the irradiated flowers produced fruits. In fact, only one of the buds produced a flower. That bud was well advanced in its development at time of the irradiation. Two months after irradiation some of the new growth which had developed, produced flowers and in 25 per cent. of the plants, fruits, often abnormal in shape, were produced. Five weeks after irradiation of plants in series V, there was a noticeable difference in height, branching habit, and fruiting condition of the control and X-rayed plants (figure 2). Buds which had been present at the time of irradiation had dropped without opening. No new buds developed until considerably later.

Data given in table IV show that irradiation of young tomato seedlings with a dose of medium intensity may delay fruit formation somewhat but in no way inhibit it on growth produced a few weeks after exposure. One medium dose given at a time when small buds are formed, causes plants to be almost completely sterile until new growth develops. A small percentage of the irradiated plants will, in time, produce fruits on the new abnormal growth.

TABLE IV

FRUIT DEVELOPMENT

SERIES NUMBER	AGE OF PLANT IRRADIATED	DOSAGE* MILLIAMPERE-MINUTES (DISTANCE 30 CM., NO FILTER)	TIME AFTER RADIATION WHEN RECORD WAS TAKEN	FRUITS PRODUCED	
				IRRADIATED	CONTROL
	days		*days*	Av. no. produced per plant	
I	17	7950	200	5.3	4.6
II	17	9000	.....	Fruits delayed	Appeared in normal time
				Percentage of plants bearing fruits	
III	17	12000	90	80	90
IV	17	6000	99	9	63.6
			107	0	27.2
			136	54.4	90.9
V	62	6000	35	0	75
			64	25	91.7
VI	60	6000	45	18	100
VII	55	6000	50	71	100

* In series I–V, 5 in. spark gap used; in VI and VII, potential of 90 K.V.

When plants are given one medium dose at an early age, fruit development is but little affected; but if this first dose is increased, fruiting is

delayed although not inhibited. In one series of plants where one medium dose was given just as buds were forming, none of the irradiated buds produced fruits. New growth which developed during the next two months, produced abnormal flowers and fruits.

In other series, where one exposure was made after buds had formed, the effect seemed to vary with the age of the buds. If buds were of fair size, they became yellowed and dropped off; but when very small at the time of exposure they produced flowers showing some anomaly of structure. If fruits developed at a later period, they were misshapen and in many cases without seeds. Figure 3 shows a cross-section view of four fruits from irradiated plants of series VII with three controls.

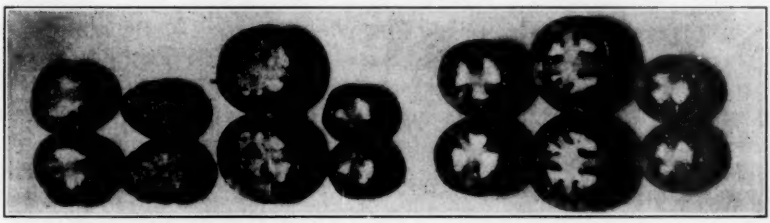

FIG. 3. Cross-sections of tomato fruits. At right, controls; at left, fruits which developed on plants which had received one medium X-ray dose after the formation of small buds. Note the absence of definite loculi, pockets in the pericarpal wall, disorganization of core and placenta, and the reduced number of seeds.

The control presents the appearance of the normal tomato fruit. The flesh consists of the outer pericarpel wall, the radial walls or interlocular septa, the placentae and the core. The number of loculi shows clearly. In the fruits from irradiated buds, however, one cannot distinguish definite loculi. The placenta and core show lack of normal structure and, in some fruits, there is an almost total absence of seeds. Pockets, which have formed in the outer pericarpel wall of the experimental fruits, are not present in the controls.

Summary

1. Irradiation of young tomato seedlings causes marked leaf anomalies in the young growth which develops after irradiation. Leaflets are often absent, badly twisted, or joined together in unusual fashion. Soon after irradiation, the leaves present a peculiar warty or pebbly appearance. As they grew older, there are areas of green of varying intensities so that they appear spotted or variegated.

2. Plants receiving X-ray dosage develop many lateral branches which cause them to assume a bushy appearance. The number of branches developed may be sixty-five per cent. greater in the irradiated plants than in the controls.

3. Many abnormalities of floral parts, including production of double blossoms, occur when plants are thrice irradiated previous to blossoming. If, however, radiation occurs at time of budding when there has been no previous dose, the buds become yellow and drop off without blossoming. Later growth may produce blossoms, some of which are normal while others are double or triple.

4. Fruit development is somewhat delayed in plants which are irradiated during their early seedling stages. However, in plants which were irradiated just before blossoming with one medium dose, complete sterility was present for some time. Later the new growth produced small abnormal fruits on twenty-five per cent. of the plants as compared with one hundred per cent. fruit production of the controls.

5. Radiophylaxis, a lessening of the effect of a harmful dose by giving a preceding lighter one, was demonstrated by tomato flowers in one series. A dose which would ordinarily cause a yellowing and dropping of buds if given as the buds were forming, did not have such a markedly fatal effect when two earlier doses were given before the reproductive stage.

6. Fruits which do develop on irradiated plants show a lack of definite internal pattern. The placenta and core show abnormal development and there is an almost total absence of seeds. Pockets formed in the pericarp of fruits from irradiated plants are not found in the controls.

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LITERATURE CITED

1. ANCEL, P., and LALLEMAND, S. Sur la protection contre l'action des rayons X par une irradiation préalable (radiophylaxie). Compt. Rend. Soc. Biol. **99**: 1588–1590. 1928.
2. JOHNSON, E. L. Effects of X-rays upon growth, development, and oxidizing enzymes of *Helianthus annuus*. Bot. Gaz. **82**: 373–402. 1926.

EFFECT OF FERTILIZERS AND DATE OF PLANTING ON THE PHYSIOLOGICAL DEVELOPMENT OF THE CORN PLANT[1]

R. W. GERDEL

(WITH TWELVE FIGURES)

Introduction

As a part of the Ohio program of corn borer research several years of investigations on the growth, rate of maturity, and yield of early and late planted corn have been carried on at Wooster. Studies have been made of the physiological conditions associated with the development of the plant, with differences in yield, and with variations in the length of time required for the maturing of late season varieties, that resulted from varying, (1) the date of planting; (2) the time and method of fertilizer application; and (3) the kind and quantity of fertilizer used.

Among the many contributions to our knowledge of the physiological and chemical phenomena of the growth of plants are numerous investigations leading to the establishment of the S-shaped curve of growth and attempting to offer an explanation of it. The papers on this subject are too numerous to review in detail.

WOLFE (**9**) found that within a given variety, the highest yielding corn plants silk early, and that the corn plants which mature first are the ones that tassel, silk, and shed pollen earliest. He concluded that any one of these characters indicates the relative time at which plants will reach final maturity. MCCLELLAND (**3**) obtained satisfactory yields when late planting of corn was followed by heavy rainfall, but if followed by a drought, the yield was greatly reduced. MCCOOL (**4**) found that the sap of corn plants grown on a soil receiving fertilizer had a greater osmotic concentration than when grown on unfertilized soils. He concluded that this greater concentration in the sap would prevent late spring frost injury to seedling corn plants.

MILLAR (**6**) obtained a more vigorous growth in corn which received hill applications of fertilizer, than in corn which received fertilizer broadcast. Early maturity also followed as a result of hill fertilization. No limitation in root development was noted as a result of placing the fertilizer in the hill. His method of applying the fertilizer was to scatter it over an area of about 5 by 10 inches, in a shallow depression, and cover with a light layer of soil before dropping the seed.

[1] Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

HARPER (**2**), who applied the fertilizer on an area of about 10 by 3 inches and 1.5 inches above the seed, found no deleterious effect from hill fertilization on the root systems of the corn plant. He suggests that very heavy applications of fertilizers high in nitrogen may produce smaller root systems.

TRUOG, *et al.* (**8**) found that germination of the seed was unimpaired when a complete fertilizer was applied at the rate of 120 to 200 pounds in the hill above the seed, and that maturity may be hastened as much as two weeks through hill applications of fertilizers on corn at planting time, providing some method is used to prevent direct contact of seed and fertilizer in the hill.

Plots

Burr-Leaming corn was grown in each of 3 years on a series of plots located on Canfield silt loam of medium to low fertility. The soil was fairly well drained and had an approximate reaction of pH 5.5. Two tons of ground limestone were applied as a basic treatment.

The series of plots comprised four separate sections, each containing twenty-eight 1/20 acre plots. Ten plots of each section were used as checks, the remaining eighteen being given different fertilizer treatments. Each year two sections with duplicate plot treatments were planted to corn; one section on or about May 15, the other on or about June 5.

The fertilizers[2] applied to the plots consisted of incomplete and complete types, such as 0–16–0, 0–12–4, and 3–12–4. Manure supplemented with 16 per cent. superphosphate was also applied to some plots. The quantity of chemical fertilizer applied varied from 100 pounds per acre, which may be considered as establishing a low level of fertility, up to 400 pounds per acre which may be considered as establishing a high level of fertility.

The fertilizers were applied to the plots at planting time either all broadcast, all in the hill, or part broadcast and part in the hill. The latter method is termed a "split application." Some of the plots received applications of 8 tons of manure and 225 pounds of 16 per cent. superphosphate broadcast. This treatment was repeated also on other plots and supplemented with varying amounts (100, 200 and 400 pounds per acre) of a 3–12–4 fertilizer applied in the hill. Hill applications were made with a modern corn planter through the fertilizer attachment which provided for some separation of seed and fertilizer.

[2] Fertilizer were mixed from nitrate of soda, 16 per cent. superphosphate and muriate of potash. A fertilizer of 3–12–4 analysis contained a quantity of nitrate of soda equivalent to 3 per cent. NH_3, superphosphate equivalent to 12 per cent. P_2O_5 and muriate of potash equivalent to 4 per cent. K_2O. Uniform volumes were obtained by using appropriate quantities of gypsum as a filler.

Physiological studies

Green and dry weights.—One end of the plots on each section was selected for uniformity of stand, and from this end of each plot two hills were removed at 10 to 14 day intervals for determination of green and dry weights. The dried sample was stored for future analysis.

Height.—Early in the season four hills in each plot were marked with a garden stake and at each sample period the height of these hills was measured and averaged for the particular plot. In 1926 and 1927 all plants were measured to the tip of the inside leaf until the tassel appeared, then to the base of the tassel. It was hoped that such a measurement would give an index of vegetative height and eliminate reproductive growth and leaf length from the final measurements. However, the variations in height of the inside leaf were too great, and to correlate the results with other work at Wooster, all measurements were made to the top of the tallest leaf in subsequent years.

Leaf area.—Measurements of leaf area were made on the samples collected for green and dry weight determinations. When small, all six plants in the sample were measured; as they became larger, the number of plants on which leaf area measurements were made was reduced to expedite handling. When measurements were made on less than the total number of plants in the sample, leaf area was determined on those plants which closely approached the average field height for that sample date. All results were calculated to a single plant basis.

Measurement of leaf area was made on a specially constructed photometer involving photo-electric cells and vacuum tube amplification. A description of the photometer used in 1927 has been published (**1**). In 1928 it was found that the errors in the method could be reduced almost one-third by using two photo-electric cells in parallel.

Vein and intervein width.—In 1928 measurements were made to determine whether intervein width of the ear leaf might give an index of the efficiency of the leaf with respect to vegetative growth and yield. Measurements were made by an ocular and stage micrometer on a cross-section of the leaf taken at a definite distance above the leaf's junction with the stalk.

Stalk diameter.—Measurement of the least and greatest thickness of the stalks from the periodic samples was made at the center of the internode above the first node, and the two figures thus obtained were averaged and designated as stalk diameter. The average diameter of all six stalks in each periodic sample was then obtained.

Mean silking date.—Beginning with the appearance of the first silks, numbered tags were placed each morning on all ears which had silked over night. At the end of the silking period these tags were collected and the mean silking date calculated, (MEYERS, **5**). Thus an index of the rate of

maturity was obtained as well as the effect of fertilizers on hastening the completion of this period in the life cycle of the plant.

Yield and moisture at harvest.—Harvest yields were determined on all plots and a 25-pound sample of ears selected at random. These samples were dried in a heated drying room and used to calculate the air-dry yields. Brown-Duvel moisture determinations were made on these samples and final yields calculated to a basis of 15.5 per cent. moisture. Marketable ears and nubbins were separated in the field at harvest, and, after drying, a shelling per cent. of the marketable ears was determined.

Results

Effect of degree of fertility upon the accumulation of dry weight

When the total dry weights from the periodic samples are plotted against time, the curves approach the S-shaped growth curve. However, as noted in figure 1 the curves for all plots receiving fertilizers exhibit two fairly well defined points of inflection. These curves would be of the bimodal type if plotted only on the actual increase in dry weight from sample to sample. Each curve apparently represents two S-shaped curves in which the autostatic end of the first curve overlaps the autokinetic end of the second (Robertson, 7).

The type of curve presented in figure 1 may be correlated with the main phases of growth of the plant, vegetative and reproductive. The vegetative phase was practically complete on the fertilized plots at the mean date of silking. In every case, where large amounts of fertilizer were applied, the average date of silking occurred a few days after senescence became prominent in the vegetative phase. This was indicated by a slowing up of the growth rate and by the inflection in the curve of growth, or, in the words of Robertson (7), "when vegetative growth attains maximal extent and minimal velocity."

From the graphs and tabular data (figures 1 and 2, and table I) it appears that hill application of fertilizer favors a more distinct separation of the vegetative and reproductive growth cycles. On the other hand, even much larger amounts of total nutrients supplied in broadcast applications of manure and superphosphate show but little of this tendency even though productive of higher final yields. Explanation apparently lies in the concentration of nutrients close to the seed in the hill treatments, this inducing more rapid early growth and earlier silking. Where hill treatment is combined with the broadcast application of manure and superphosphate, growth is rapid during both growth phases with greatest advance in silking date, and the two growth phases show distinct separation.

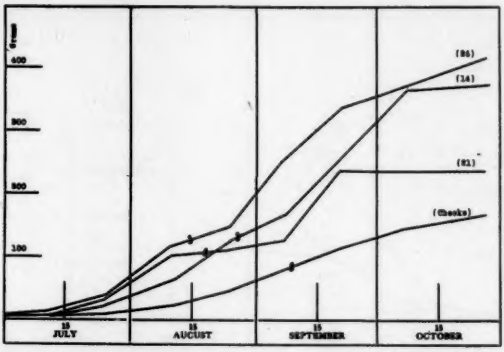

FIG. 1. Average dry weights of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(26) Manure 8 tons, 0–16–0 225 lb. broadcast, 3–12–4 200 lb. hill.

(14) Manure 8 tons, 0–16–0 225 lb. broadcast.

(21) 3–12–4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

COMPARISON OF DRY WEIGHTS FOR 1926, 1927, AND 1928

The data so far presented apply only to the 1927 crop. The dry weight curves for 1926, 1927, and 1928 all follow the same general trends as shown in figure 3. Seasonal variation in meteorological conditions cause differ-

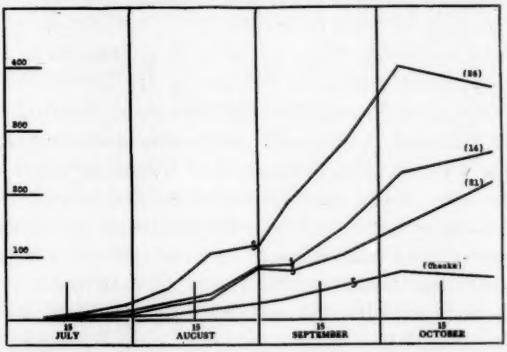

FIG. 2. Average dry weights of plants grown on plots receiving varying types of fertilizers. Planted June 5, 1927.

(26) Manure 8 tons, 0–16–0 225 lb. broadcast, 3–12–4 200 lb. hill.

(14) Manure 8 tons, 0–16–0 225 lb. broadcast.

(21) 3–12–4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

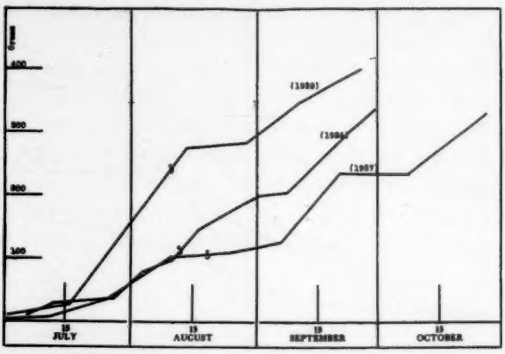

FIG. 3. Average dry weights of plants grown on all plots receiving hill applications of 3–12–4 in 1926, 1927. and 1928.
S = mean silking date.

ences in both the vegetative and reproductive phases of growth; hence, the averaging of data for two or more years would obscure the effects due to variations in climatic factors. The average date of silking varies by more than 6 days; evident senescence of vegetative growth varies by at least 20 days, while frost, sufficiently early and intense in 1926 and 1928 curtailed the reproductive growth sufficiently to prevent a complete expression of this portion of the cycle. The 1927 results, therefore, are more complete and were selected to indicate the general trends of growth as shown by dry weight accumulation.

The effects of variations from the normal in temperature and rainfall throughout the growing season on increase in dry matter are presented in figure 4. In 1928 an extremely wet and cold June resulted in the storage of considerable moisture in the soil. July was warm with about normal precipitation; as a result, a large quantity of nitrate nitrogen was probably produced in the soil. These favorable conditions of moisture, temperature, and available nitrogen were directly reflected in the rapid growth at this time, as contrasted with that during the same period in 1927 which was undoubtedly inhibited as the result of cold, dry weather in June. July of 1927 was about normal but not sufficiently favorable to make up for the June deficiency in temperature and rainfall. In 1928 an extremely warm August with more than normal precipitation provided conditions favorable to rapid growth, while the opposite seasonal conditions occurred in 1927. This causes the dry weight curves for these two years to diverge widely at this time. However, an early frost and a cold dry autumn stopped all growth before the end of September in 1928, while more favorable conditions permitted the corn to grow at least thirty days longer in 1927.

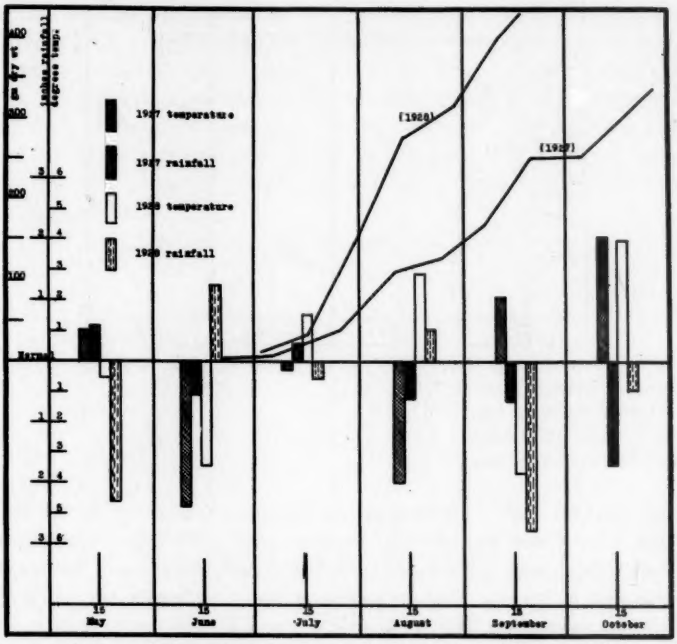

FIG. 4. Correlation of dry weight increase with seasonal variation in climatic factors. Average of all plots receiving hill applications of 3–12–4.

EFFECT OF DEGREE OF FERTILITY ON STALK DIAMETER, LEAF AREA, HEIGHT, AND SILKING DATE

Figure 5 shows the average stalk diameter of corn at different degrees of fertility. The most interesting observation is the closeness of the maximum diameter to a single value for all three plots, indicating that Burr-Leaming corn has a seasonal maximum diameter which it reaches with comparatively low fertility. The higher the degree of fertility, the more rapid was the early growth and the attainment of this maximum stalk diameter. The fact that Burr-Leaming corn attains a seasonal maximum diameter at comparatively low levels of fertility is further indicated in figure 9 in which the average maximum diameter for 1927 and 1928 from twelve different plots is plotted against the yield produced at increasing degrees of fertility.

The leaf area curves for the plots receiving 3–12–4 fertilizer in the hill in 1926, 1927, and 1928 are presented in figure 6. The most striking thing about these curves is the rapid acceleration in leaf area development during

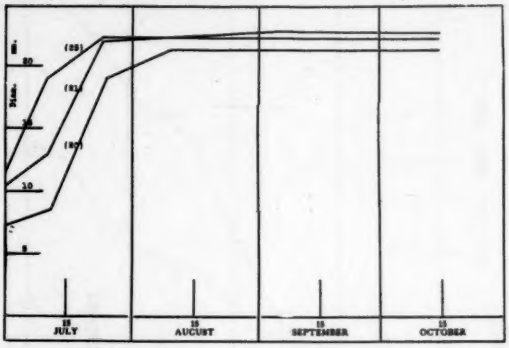

FIG. 5. Average stalk diameter of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.
(23) 3–12–4 400 lb. hill.
(21) 3–12–4 200 lb. hill.
(20) 3–12–4 100 lb. hill.

the early part of 1928. There may have been an initial lag in the acceleration, but if so it was too small to be measured within the sample periods. However, though acceleration is rapid in the early stages and the maximum area attained is greater than in previous years, a longer time is required to reach this maximum leaf area.

The 1927 data are used for comparing the effects of fertility treatments upon increase of leaf area, the effects of manure and superphosphate alone, complete fertilizer alone, and the combination of these two fertilizers being shown in figure 7.

It is apparent that a seasonal maximum leaf area may be obtained by the use of fertilizers. This limitation of leaf area is similar to that observed for stalk diameter. The three early planted, fertilized plots shown in figure 7 represent a wide variation in fertility; yet the maximum leaf area attained by any one of these plots varies by less than 200 square centimeters from that of either of the others. The maximum variation between the fertilized plots is approximately 4.5 per cent. while any one of these three plots varies from the unfertilized check plot by 2,000 cm.^{2}, or 45 per cent. Further evidence of the production of maximum leaf area at relatively low degrees of fertility is shown in figure 9, in which the two year average maximum leaf area is compared with the yield at increasing levels of fertility.

From observation of figure 1 it is noted that the increase in dry weight for these plots is apparently independent of either leaf area or stalk diameter. This may indicate that the nutrients are first utilized in the

growth of the stalk and leaves up to a certain size. If these nutrients are just sufficient to permit maximum attainment of vegetative growth for a given season, little is left for reproductive growth. Where fertilizers are added to the soil and absorbed in excess of the requirements for vegetative processes, the excess becomes available for continued reproductive growth. This relationship is further indicated by the data presented in table II, showing the leaf areas and yields at several degrees of fertility for the years 1927 and 1928.

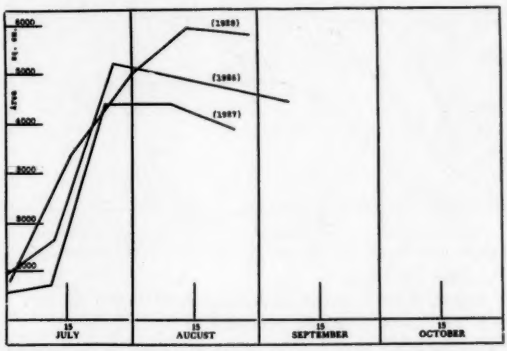

FIG. 6. Average leaf area of plants grown on all plots receiving hill applications of 3–12–4 in 1926, 1927, and 1928. Planted about May 15, each year.

In the analysis of these data it must be remembered that a single leaf may have an area of 200 to 300 cm.^{2} or even more. The loss of a single leaf, due to drying and breaking off, would affect the leaf area of the plants sampled by a considerable amount. This loss of leaves occurring earlier in the early planted corn accounts at least in part for the greater area of leaves in the last samples of late planted, heavily fertilized corn. However, the data as a whole substantiate the previous hypothesis that for Burr-Leaming corn a maximum leaf area is attained at fairly low concentrations of fertilizer.

The maximum leaf area attainable in any season is determined in part by meteorological conditions, such as rainfall and temperature. For the years 1926 and 1927 the maximum area reached varied between 4500 and 5000 cm.^{2}; while for the year 1928 it was 6500 to 7000 cm.^{2} The latter year was characterized by heavy rainfall and high temperatures. However, the relative effects of fertilizers upon attainment of maximum leaf area for the season was about the same in all three years, a fairly low degree of fertility being capable of producing a maximum leaf area equal to that attained by the heaviest fertilized corn.

Apparently, from a study of the data in tables I and II and the figures 7, 8 and 9, neither maximum leaf area nor time of its attainment are indices of potential yield for every degree of fertility. Below that degree of fertility at which maximum leaf area is attained, yield and leaf area decrease

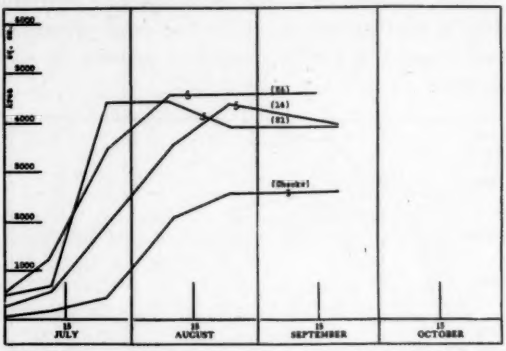

FIG. 7. Average leaf area of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(26) Manure 8 tons, 0–16–0 225 lb. broadcast, 3–12–4 200 lb. hill.

(14) Manure 8 tons, 0–16–0 225 lb. broadcast.

(21) 3–12–4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

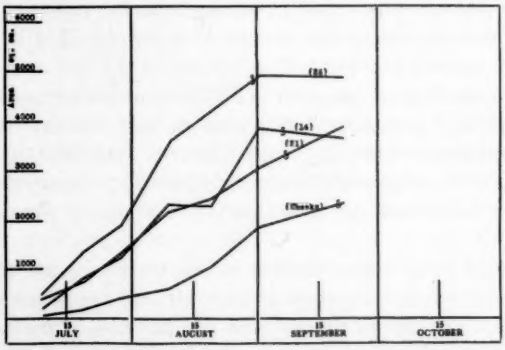

FIG. 8. Average leaf area of plants grown on plots receiving varying types of fertilizers. Planted June 5, 1927.

(26) Manure 8 tons, 0–16–0 225 lb. broadcast, 3–12–4 200 lb. hill.

(14) Manure 8 tons, 0–16–0 225 lb. broadcast.

(21) 3–12–4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

almost proportionally; above this degree of fertility, yield increases very rapidly while leaf area remains constant.

TABLE I

YIELD OF CORN AS AFFECTED BY DEGREE OF FERTILITY

PLOT	TREATMENT	PLANTED MAY 15, 1927			PLANTED JUNE 5, 1927		
		YIELD			YIELD		
		EARS	NUBS	TOTAL	EARS	NUBS	TOTAL
		bu.	*bu.*	*bu.*	*bu.*	*bu.*	*bu.*
26	manure, 8 T., 0–16–0 225 lb. Bc., 3–12–4 200 lb. hill	55.6	14.7	70.3	39.5	8.4	47.9
14	manure, 8 T., 0–16–0 225 lb. Bc.	27.9	17.3	45.2	14.3	17.6	31.9
21	3–12–4 200 lb. hill	23.8	13.4	37.2	11.4	13.2	24.6
Checks	unfertilized	4.7	12.5	17.2	1.3	3.5	4.8

In plotting the curves in figure 9 the fertilized plots were arranged in order of yield, and not always in order of increasing fertilizer application. Often the method of application was just as effective in determining the fertility level and yield on the plots as the quantity applied.

The vein and intervein widths of ten leaves removed at the ear node from each of three different plots were measured at a point 10 cm. above the junction of the leaf with the ear. The results are presented in table III.

Although there is a slightly greater intervein width in the leaves of corn from highly fertilized plots, the difference is so small as to be practically negligible when contrasted with the great differences in yield.

Since seasonal maximum leaf area is reached at relatively low fertility; and, as there is almost no increase in the intervein vein ratio, it would appear that the attainment of the maximum efficiency of the leaves of Burr-Leaming corn is limited by the nutrients available in the soil.

The general trend of increase in height throughout the growing season is affected by fertility in a manner quite similar to either dry weight, leaf area, or stalk diameter. (See figures 9, 10, and 11.) However, the final height apparently is not so much affected by internal physiological conditions as are leaf area and stalk diameter; that is, though there is probably a genetic and physiological limitation to final height beyond which the degree of fertility will not produce greater elongation of the stem, this limitation

TABLE II

LEAF AREA AND YIELD OF THE CORN PLANT AS AFFECTED BY THE DEGREE OF FERTILITY

Treatment	Planted May 15						Planted June 5					
	Maximum leaf area			Yield			Maximum leaf area			Yield		
	1927	1928	Av.	1927	1928	Av.	1927	1928	Av.	1927	1928	Av.
	cm.²	*cm.²*	*cm.²*	*bu.*	*bu.*	*bu.*	*cm.²*	*cm.²*	*cm.²*	*bu.*	*bu.*	*bu.*
Manure, 8 T. 0–16–0 225 lbs. broadcast 3–12–4 200 lb. hill	4510	7032	5771	70.3	85.2	77.7	4960	7965	6462	47.9	80.5×	64.2
Manure, 8 T. 0–16–0 225 lb. broadcast	4343	6953	5648	45.2	75.0	60.1	3877	6520	5198	31.9	29.7+	30.8
3–12–4 400 lb. hill	4565	6970	5768	44.2	63.7	53.9	5057	7259	6158	25.1	44.0	34.5
3–12–4 200 lb. hill	4484	5917	5151	37.2	63.5	50.3	3820	6615	5217	24.6	48.2	36.4
3–12–4 100 lb. hill	3678	5232	4455	27.9	66.5*	47.2	3180	5965	4572	19.6	45.0	32.3
Checks, unfertilized	2833	4770	3801	20.3	43.1	31.7	2317	5234	3775	6.7	32.1	19.4

* Adjacent check yielded 55.2 bushels per acre.

+ Adjacent check yielded 13.7 bushels per acre.

× Yield calculated from less than total plot area after damage by birds.

TABLE III

VEIN AND INTERVEIN WIDTH OF CORN LEAVES

TREATMENT	WIDTH		RATIO
	AV. OF TEN LEAVES		INTERVEIN
	VEIN	INTERVEIN	VEIN
	mm.	*mm.*	
Manure, 8 T., 0–16–0 225 lb. broadcast 3–12–4 200 lb. hill	0.10166	101.2	1/995
3–12–4 200 lb. hill	0.09243	91.4	1/988
Checks, unfertilized	0.08466	81.7	1/965

is not effective at such low fertility levels as seemed to cause the expression of the internal limiting factors for leaf area or stalk diameter.

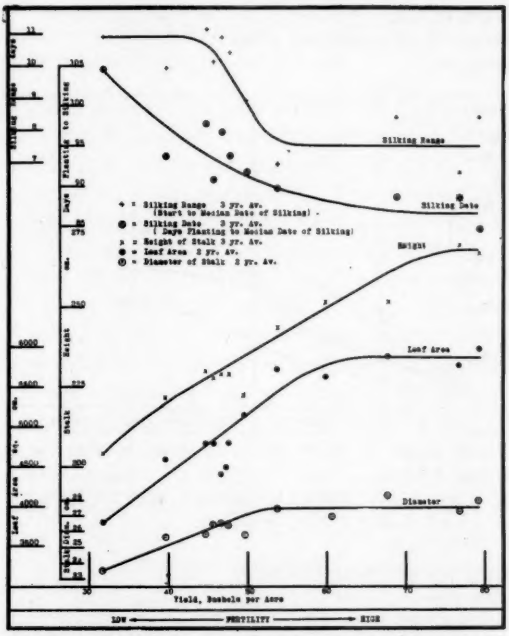

FIG. 9. Effect of degree of fertility on the yield and physiological development of the corn plant.

The failure of the lower fertilized plots to reach heights even approximately equal to those of the higher fertilized plots is pronounced, as is

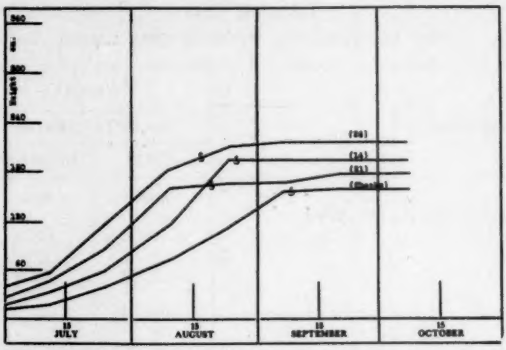

FIG. 10. Average height of plants grown on plots receiving varying types of fertilizers. Planted May 15, 1927.

(26) Manure 8 tons, 0–16–0 225 lb. broadcast, 3–12–4 200 lb. hill.

(14) Manure 8 tons, 0–16–0 225 lb. broadcast.

(21) 3–12–4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

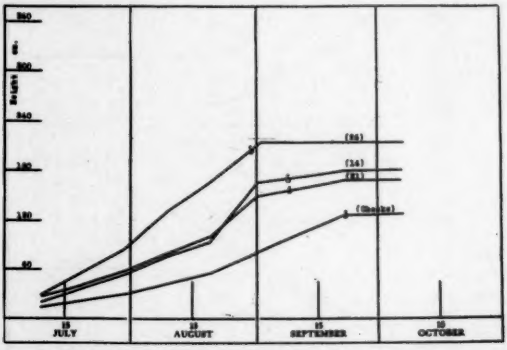

FIG. 11. Average height of plants grown on plots receiving varying types of fertilizers. Planted June 5, 1927.

(26) Manure 8 tons, 0–16–0 225 lb. broadcast, 3–12–4 200 lb. hill.

(14) Manure 8 tons, 0–16–0 225 lb. broadcast.

(21) 3–12–4 200 lb. hill.

(Checks) average of all unfertilized plots.

S = mean silking date.

also the fact that the highly fertilized plots for both early and late plantings produce corn of nearly equal height. This would indicate that this height of approximately 225 centimeters is probably the limit of elongation for that particular year. Further evidence of the fact that the maxi-

mum potentiality for height is expressed only at high degrees of fertility is given in table IV. The fact that height is limited only at high levels of

TABLE IV

HEIGHT OF THE CORN PLANT AS AFFECTED BY THE DEGREE OF FERTILITY

TREATMENT	FINAL HEIGHT			
	1927*	1928×	1929×	AV.
	cm.	*cm.*	*cm.*	*cm.*
Manure, 8 T., 0–16–0 225 lb. broadcast 3–12–4 400 lb. hill	225	318	260	267.6
Manure, 8 T., 0–16–0 225 lb. broadcast 3–12–4 200 lb. hill	215	330	265	270.0
Manure, 8 T., 0–16–0 225 lb. broadcast	195	308	255	252.6
3–12–4 200 lb. hill	170	265	227	221.0

* 1927 height measured to base of tassel.

× 1928 and 1929 height measured to top of tassel.

fertility is shown in figure 9 in which the three year average maximum height is compared with yield for increasing degrees of fertility.

The value of the date of silking as an index of various physiological phenomena can scarcely be over-emphasized. The method of obtaining the mean date of silking has been previously discussed by MEYERS (5).

The mean silking date occurs almost always at the time when maximum vegetative growth is attained, as shown in all of the previous graphs. In some cases, this point falls slightly ahead of the maximum attainment of height or leaf area, in others it occurs after these maxima have been reached. Photoperiodism is almost certain to play an important rôle in the determination of the time of silking, but it cannot completely overcome the handicap of poor environment, late planting, and lack of varietal adaptation.

Although photoperiodism may play an important part in the determination of the flowering time of plants, in the corn plant silking exhibits sufficient variability in time of occurrence to be influenced by fertility to an extent which is not only measurable but of economic importance (tables V and VI).

The most notable effects were produced by hill applications of complete fertilizer supplementing broadcast applications of manure and superphosphate. Such treatment not only caused earlier silking but also reduced the length of the silking period. In 1927 the plot receiving this treatment reached the mean date of silking 24 days earlier and completed the silking

TABLE V

AVERAGE SILKING DATE AND YIELD OF CORN PLANT AS AFFECTED BY DEGREE OF FERTILITY. PLANTED MAY 15

TREATMENT	PLANTING TO SILKING		SILKING PERIOD*	YIELD, 15.5 PER CENT. MOISTURE				MOISTURE AT HUSKING	
				1927		3 YEAR AV.			
	1927	3 YEAR AV.	1927	MARKETABLE	TOTAL	MARKETABLE	TOTAL	1927	3 YEAR AV.
	days	*days*	*days*	*bu.*	*bu.*	*bu.*	*bu.*	*per cent.*	*per cent.*
Manure, 8 T. 0–16–0 225 lb. } broadcast 3–12–4 200 lb. hill	92	89	7	55.6	70.3	68.8	77.7	33.6	35.1
Manure, 8 T. 0–16–0 225 lb. } broadcast	104	98	11	27.9	45.2	51.8	65.5	46.7	42.0
3–12–4 400 lb. hill	93	90	7	30.4	41.2	47.8	58.4	36.9	36.8
3–12–4 200 lb. hill	97	92	9	23.8	37.2	43.5	55.1	36.9	36.7
3–12–4 100 lb. hill	104	97	11	14.2	27.9	41.8	52.8	41.9	40.0
Checks unfertilized	116	105	11	6.5	20.3	24.7	39.2	46.7	45.1

* Number of days from start of silking to average date of silking.

TABLE VI

AVERAGE SILKING DATE AND YIELD OF CORN PLANT AS AFFECTED BY DEGREE OF FERTILITY. PLANTED JUNE 5

TREATMENT	PLANTING TO SILKING		SILKING PERIOD*	YIELD, 15.5 PER CENT. MOISTURE				MOISTURE AT HUSKING	
				1927		3 YEAR AV.			
	1927	3 YEAR AV.	1927	MARKETABLE	TOTAL	MARKETABLE	TOTAL	1927	3 YEAR AV.
	days	*days*	*days*	*bu.*	*bu.*	*bu.*	*bu.*	*per cent.*	*per cent.*
Manure, 8 T. 0–16–0 225 lb. broadcast 3–12–4 200 lb. hill	78	88	11	39.5	47.9	.....	.....	50.1	.....
Manure, 8 T. 0–16–0 225 lb. broadcast	87	83	10	14.3	31.9	20.9	36.9	53.5	55.0
3–12–4 400 lb. hill	84	82	10	13.2	22.9	22.4	42.3	51.9	48.4
3–12–4 200 lb. hill	87	84	10	11.4	24.6	26.1	42.9	42.6	43.6
3–12–4 100 lb. hill	90	86	9	7.2	19.6	24.1	34.9	54.3	49.0
Checks unfertilized	99	93	9	1.8	6.7	16.1	28.5	67.5	58.2

* Number of days from start of silking to average date of silking.
Three year average unavailable for first plot in this table.

period in 8 days less time than the unfertilized plots. Further comparison of the data shows that as the quantity of chemical fertilizer is decreased the time to reach the average date of silking becomes greater and the time for completion of silking is increased.

The importance of method of application of nutrients, in this connection, is shown by the results for the plot receiving manure and superphosphate without supplementary hill treatment. On the basis of its yield and of the results obtained by WOLFE (9) previously discussed, this plot should silk earlier than any of the plots receiving only complete fertilizer. In all other cases the higher the yield, the earlier occurred the mean date of silking. The failure of this plot to silk earlier may be accounted for by the slow initial growth as demonstrated by the leaf area, height, and dry weight curves for this treatment. It is also evident from the data in tables V and VI that late silking is correlated with high moisture content in the grain at harvest, a factor of considerable economic importance since moisture content is the most important factor determining the grade of market corn.

The effect of fertility upon the rate and duration of silking is presented graphically in figure 12. The rapidity of silk development with high fertility and the slow development and the long drawn-out blooming period with low fertility are emphasized in this graph.

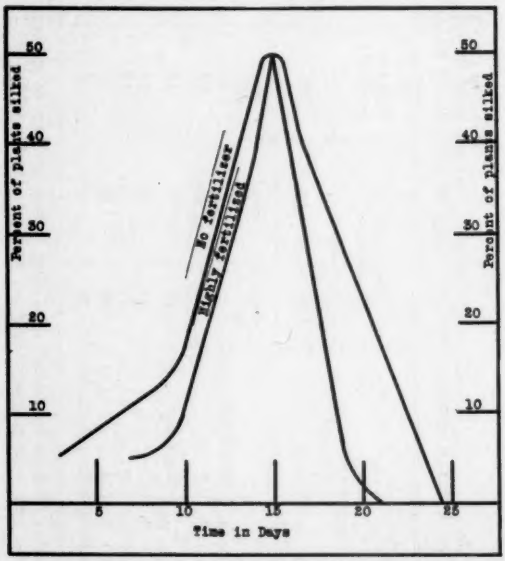

FIG. 12. Rate of silking as affected by fertility.

That earliness of silking and shortening of the duration of the silking period are limited by fertility level as are leaf area, height and diameter is shown in figure 9.

Low concentrations of fertilizer are not effective in shortening the silking period, but at medium concentrations the duration of the silking period is rapidly shortened to the minimum attainable with Burr-Leaming corn. The earliest median date of silking, however, is reached only at relatively high levels of fertility.

Probably the silking phenomena are greatly influenced by the response of individuals in the heterogeneous population of these plots. Low fertility levels appear to affect the silking phenomena in a manner similar to dry weight accumulation, for those plots which exhibit the longest duration of the silking period, and which reach the average date of silking late in the season, also exhibit the most complete mergence of the growth and reproductive cycles as shown by the smooth, single sigmoid curve for dry weight accumulation.

A conclusion of considerable practical value that may be drawn from a study of the data for silking date, yield, and moisture at harvest is the possibility of maturing an otherwise unadapted, long-season variety of corn by the use of sufficient fertilizer applied in the hill.

Summary

Periodic measurements of the physiological development of the corn plant were made on corn grown on plots representing 19 different degrees of fertility and two different dates of planting.

Periodic measurements on samples collected at ten- to fourteen-day intervals included green weight, dry weight, stalk diameter, and leaf area.

Field measurements included height, silking date, and yield of grain. The moisture content of the grain at harvest was also determined.

A summary of the results follows:

1. With continuous abundance of nitrogen, phosphorus, and potassium throughout the season, marked differentiation of the vegetative and reproductive cycles occurred, associated with a narrow silking range, earliness of silking, and high yield.

2. With a low supply of nitrogen, phosphorus, and potassium throughout the season, an overlapping and mergence of the two growth cycles occurred, associated with a wide silking range, lateness of silking, and low yield.

3. Hill applications of fertilizers appeared to intensify the differentiation of the vegetative and reproductive cycles. Broadcast applications were markedly less effective, presumably because less nutrients were concentrated within reach of the plant during its early period of growth.

4. Maximum leaf area, stalk diameter, and height of the corn plant were produced at levels of fertility considerably below that which still produced increase in yield.

5. The narrowest silking range and earliest average date of silking occurred at levels of fertility considerably lower than that which still produced increase in yield.

6. The degree of fertility at which the different physiological maxima were obtained was approximately the same from year to year, regardless of the annual fluctuation in maxima due to seasonal conditions.

7. Variations in the physiological development of corn from different levels of fertility may represent, under the conditions of this experiment, a variable response of individuals in the heterogeneous population of Burr-Leaming corn.

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LITERATURE CITED

1. GERDEL, R. W., and SALTER, R. M. Measurement of leaf area using the photo-electric cell. Jour. Amer. Soc. Agron. **20**: 635–642. 1928.
2. HARPER, H. J. A study of the secondary effects of hill fertilization. Iowa Agr. Exp. Sta. Res. Bull. 87. 1925.
3. MCCLELLAND, C. K. Effect of different dates of planting corn on yields. Arkansas Agr. Exp. Sta. Bull. 222. 1927.
4. MCCOOL, M. M. Relation of soil to plant cell sap. Michigan Agr. Exp. Sta. Quart. Bull. **9**: 60–67. 1926.
5. MEYERS, M. T. Determining the date of silking in experiments with corn. Amer. Soc. Agron. **22**: 280–283. 1930
6. MILLAR, C. E. Hill fertilization of corn. Mich. Agr. Exp. Sta. Quart. Bull. 5: 69–73. 1922.
7. ROBERTSON, T. B. The chemical basis of growth and senescence. Chapt. III. Lippincott, Philadelphia. 1923.
8. TRUOG, E., HARPER, H. J., MAGISTAD, O. C., PARKER, F. W., and SYKORA, J. Fertilizer experiments. Wisconsin Agr. Exp. Sta. Res. Bull. 65. 1925.
9. WOLFE, T. K. A study of germination, maturity, and yield in corn. Virginia Agr. Exp. Sta. Tech. Bull. 30. 1927.

COMPARISON OF TWO METHODS OF MEASURING STOMATAL APERTURE

ERIC ASHBY[1]

(WITH ONE FIGURE)

Both England and America have contributed valuable data to our knowledge of stomatal movement in plants; and it is of interest that totally different methods have been used in the two countries. In England the porometer, first described by DARWIN and PERTZ (**2**) was modified and improved by KNIGHT (**4**), who used the method for his work on stomatal movement (**5, 6**). In the United States the most important contributions have been made by LLOYD (**7**), E. B. SHREVE (**9**), and LOFTFIELD (**8**). LLOYD originated the method of tearing off the epidermis, and plunging it immediately into absolute alcohol, subsequently measuring the stomatal aperture under the microscope. This method was also adopted by LOFTFIELD. SHREVE described a method depending upon rapid penetration by picric acid, and subsequent microscopic examination.

KNIGHT, though he mentions LLOYD'S method, has published no comparison of the method with his own. LOFTFIELD rejects the porometer method. "To the best of the writer's knowledge, no comparison has been made between a series of direct observations upon the condition of the stomata and porometer readings made at the same time. Until the method is checked in this manner it is felt that its reliability is questionable." (**8**, page 8.)

Both LLOYD'S method and KNIGHT'S have their draw-backs, which it is not the writer's purpose to discuss here. A brief comparison of the two methods may not be out of place, especially as the British method could be applied on the same species as LLOYD used, and in the same laboratory as that in which LLOYD devised his method.

For the tests two plants were used, geranium, growing in the greenhouse of the desert laboratory, and *Verbena*, growing in the garden. A porometer, similar to that described by KNIGHT (**4**), was fixed on one leaf of the plant. Readings were taken on the porometer at regular intervals. Every time a reading was taken, a strip of epidermis was removed from a leaf of about the same age, and put into a solution of congo red in absolute alcohol. The dimensions were measured under the microscope, and the areas of the pores calculated, on the assumption that the pores were elliptical in outline. In table I, and figure 1 are given the readings from

[1] Commonwealth Fund Fellow.

one experiment on a geranium leaf, in full, and in table II are readings in full made on a leaf of *Verbena*.

It will be noticed that the porometer readings are compared with the areas of the pores, and not the diameters. While diffusion is proportional to the diameters of the pores, the movement of gas under pressure is proportional to the areas.

The tables reveal certain facts relating to the relative sensitiveness of the two methods. LLOYD himself mentions that "the stomata in a piece of epidermis are seldom of a uniform degree of opening." (7, page 28.); but his method of estimating the mean stomatal aperture by measuring the extremes of aperture is not statistically sound. When the mean of ten stomatal apertures is taken, the coefficients of variability are of the order of 20 per cent. The porometer averages the apertures of some 50,000 stomata, and the coefficient of variability of five readings is as low as 3.2 per cent. Assuming that it measures stomatal aperture, the porometer, then, is a far more sensitive test of changes of aperture than LLOYD'S method. It suffers, however, from the disadvantage that absolute values cannot be obtained from the method.

TABLE I

STOMATAL MOVEMENT ON GERANIUM LEAF THROUGHOUT THE DAY

TIME	POROMETER METHOD	LLOYD'S METHOD, AREA OF PORE
	drops per minute	*mm.²*
9:30 A. M.	porometer put on	.....
10:00	7.6 ± 0.24	.....
11:00	8.1 ± 0.26	$0.000118 \pm 10^{-5} \times 2.17$
12:00	8.0	.....
1:00 P. M.	7.8 ± 0.26	$0.000116 \pm 10^{-5} \times 2.47$
2:00	7.4 ± 0.22	$0.000095 \pm 10^{-5} \times 1.82$
3:00	6.4 ± 0.20	$0.000086 \pm 10^{-5} \times 1.70$
4:00	6.9 ± 0.20	$0.000077 \pm 10^{-5} \times 1.15$
5:00	6.0 ± 0.19	$0.000079 \pm 10^{-5} \times 2.49$
6:00	2.8 ± 0.02	$0.000046 \pm 10^{-5} \times 0.73$
7:00	2.0 ± 0.02	0.000000

To test the closeness of agreement of the two methods, the two sets of readings are arranged as percentages of their maximum values, and the series of differences between the percentages compared, using the statistic χ^2, and the tables in FISHER'S monograph (3). This has been done in full for the geranium leaf (table III), and in table IV are given the results of tests on various other leaves.

TABLE II

STOMATAL MOVEMENT ON *Verbena* LEAF THROUGHOUT THE DAY

TIME	POROMETER	LLOYD'S METHOD
	drops per minute	*mm.*^{2}
9:30 A. M.	porometer put on	.....
11:00 A. M.	4.8	$0.000090 \pm 10^{-5} \times 1.8$
12:00	4.5	$0.000082 \pm 10^{-5} \times 1.6$
3:00 P. M.	2.0	$0.000048 \pm 10^{-5} \times 0.9$
6:00 P. M.	1.1	$0.000015 \pm 10^{-5} \times 0.7$

It is clear that the methods do not give results which differ significantly, except at very small stomatal apertures. At 7:00 P. M. the stomata appeared closed when examined by LLOYD'S method under the microscope, but the porometer still gave readings. This was almost invariably observed. It is not surprising that air can still be drawn through the stomata slowly after they appear to be closed. At such apertures, however, diffusion through the pores is proportional to the cube or sixth root of the rate of flow under pressure, so that LLOYD'S method gives the more accurate picture of the diffusive capacity of stomata at small apertures.

TABLE III

χ^2 TEST OF DIFFERENCES BETWEEN THE TWO METHODS ON GERANIUM

TIME	POROMETER READING IN PERCENTAGE OF MAXIMUM	LLOYD'S METHOD IN PERCENTAGE OF MAXIMUM	DIFFERENCE	χ^2
	per cent.	*per cent.*		
11:00	100	100	0	0
1:00	96.3	98.0	1.7	0
2:00	91.4	81.0	10.4	0.62
3:00	79.0	73.1	5.9	0.48
4:00	85.2	65.0	20.2	6.28
5:00	74.1	66.2	7.9	0.94
6:00	34.7	39.3	4.6	0.54
7:00	24.7	00.0	24.7	(610.1)

Total, omitting last fig. = 8.86
n ..... = 5
P ..... = 0.10–0.20

The tables reveal a very satisfactory agreement between KNIGHT'S method and LLOYD'S method of estimating stomatal apertures, on geranium and *Verbena*, at wide apertures. If the small values, taken about sunset.

TABLE IV

SUMMARY OF TESTS OF AGREEMENT BETWEEN THE TWO METHODS

NUMBER	PLANT	χ^2	n	P	DIFFERENCE
1	*Geranium*	8.86	5	0.10–0.20	not significant
2	"	7.68	4	0.10	" "
3	*Verbena*	5.40	4	0.30–0.20	" "
4	"	9.00	5	0.10	" "
5	"	5.02	4	0.30–0.20	" "

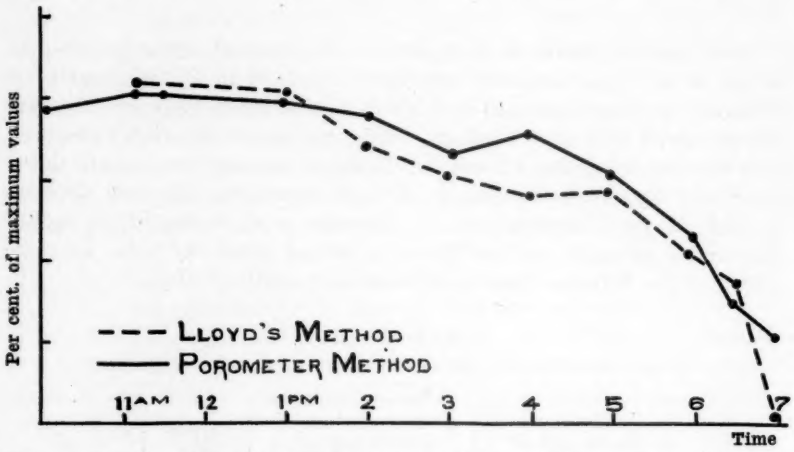

FIG. 1. Diurnal changes in stomatal aperture of the geranium, determined by two different methods.

are included, the values of χ^2 are 618.9, 442.3, 330.2, instead of 8.86, 7.68, and 2.29 and the divergence is in every case significant. Neither of these plants has heterobaric leaves.

According to the coefficients of variability of the two methods, the porometer method is nearly ten times as sensitive as the method devised by LLOYD.

In conclusion I wish to record my indebtedness to Dr. SHREVE, and my thanks to the Carnegie Institution for the facilities of the Desert Laboratory.

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LITERATURE CITED

1. Brown, H. T. and Escombe, F. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Roy. Soc. Lond. **193** B: 223–291. 1900.
2. Darwin, F. and Pertz, D. F. M. On a new method of estimating the aperture of stomata. Proc. Roy. Soc. Lond. **84** B: 136–154. 1911.
3. Fisher, R. A. Statistical methods for research workers. 3rd ed. London. 1930.
4. Knight, R. C. A convenient modification of the porometer. New Phyt. **14**: 212–216. 1915.
5. ————. On the use of the porometer in stomatal investigation. Ann. Bot. **30**: 57–76. 1916.
6. ————. The interrelations of stomatal aperture, leaf water-content, and transpiration rate. Ann. Bot. **31**: 221–240. 1917.
7. Lloyd, F. E. The physiology of stomata. Carnegie Inst. Washington. Pub. no. 82. 1908.
8. Loftfield, J. V. The behaviour of stomata. Carnegie Inst. Washington. Pub. no. 314. 1921.
9. Shreve, E. B. The daily march of transpiration in a desert perennial. Carnegie Inst. Washington. Pub. no. 194. 1914.

MECHANISM OF ROOT-CONTRACTION IN *BRODIAEA LACTEA*

D. THODAY

In a recent paper in the American Journal of Botany, F. H. SMITH (**7**) has given an interesting account of the contractile roots of *Brodiaea lactea*. These show features that clearly differentiate them from the ordinary monocotyledonous types of contractile root which the work of RIMBACH (**4, 5, 6**) has made familiar. In the latter types the stele remains straight, owing to the fact that the taut contractile tissue lies just outside it. The cells of this active tissue, the inner cortex, grow wider and shorter, as described by DE VRIES for other types (**13**). Room for their transverse growth is obtained in many cases by progressive collapse of the outer cortical cells. In *Brodiaea lactea* the outer cortex does not collapse in this way and in the later stages of contraction the stele becomes greatly distorted, which shows that the tissue immediately surrounding it cannot be actively contracting and so longitudinally taut. According to SMITH'S account it seems that there may be two phases of contraction, the first, during which the stele remains straight, brought about in the ordinary way by growth contraction of the cortex while the root is still swelling; the second, after swelling is complete, involving the distortion of the stele and progressive *collapse of transverse zones* of the cortex.

The alternation of turgid with collapsing transverse zones parallels in so remarkable a way the phenomena described by the writer for the Dicotyledon, *Oxalis incarnata* (**9**), that it seems worth while to draw attention to the similarity and to suggest that the explanation offered of the mechanism of the one may be applicable to the other.

SMITH'S explanation of the later phase of contraction is not convincing. He appears to attribute it to the distortion of the stele in consequence of the unequal distribution around it of turgid growing cells exerting on it unequal lateral pulls. The turgid layers, owing to the loss of turgor of layers above and below, are released from vertical pressure, round off and so diminish their transverse dimensions and pull on the stele. If this were the explanation the stele would be under longitudinal tension. Transverse components of this tension would be borne by the turgid tissue pulling on the convexities of the stele. If, however, the stele is compressed during the first phase of contraction, it is less likely to offer resistance to extension. Besides, a glance at the violence of its distortion makes it impossible to regard it as a taut cord. SMITH refers to the tearing of the tissue just out-

side the stele and states that it is mostly the collapsed cells that are torn loose. His fig. 7, however, shows that tearing is not confined to them.

The salient facts for an understanding of the mechanics of the situation are the following: (1) *The stele is being distorted under longitudinal compression:* the tearing of the inner cortex seems adequately accounted for as consequential upon this distortion. (2) The turgid transverse layers "round off again, increasing somewhat in length and decreasing in their radial and tangential measurements. Thus from this stage on, as the root contracts, the diameter of the root as a whole decreases." That is, *the volume of the root is diminishing* by longitudinal and to a smaller extent by transverse contraction, so that *sap must be passing out* of it—mainly it is clear, *from the collapsing cells.*

The parallel with *Oxalis incarnata* is very close. In both cases the contraction overcomes the resistance of a central core, including lignified elements, which yields by violent distortion. In neither can any tissue be found which has the continuity essential to an active contractile tissue, which must be capable itself of bearing longitudinal strain. In both cases transverse zones of collapsing tissue determine that the diminution of volume shall affect mainly the length of the organ, while intervening turgid zones prevent transverse collapse.

The explanation of the contraction thus turns on the evacuation of the collapsing cells. (*Cf.* **9,** pp. 575–576.) If these cells lose their protoplasm, as they do in *Oxalis incarnata,* and no longer retain their solutes, the existence of a water-deficit elsewhere in the plant will lead to a withdrawal of their water. In a transpiring plant this is the normal condition. Very rarely can the cells of land plants be fully turgid. The maintenance of the transpiration stream ordinarily involves the maintenance of a gradient of water deficit and a gradient of suction pressure (**1, 3, 7, 10, 11, 12**).

If now we imagine that a living cell in equilibrium with its neighbors, *i.e.* with equal suction pressure (**10**), is replaced by a cell-wall full of pure water, the equilibrium is disturbed. The wall of this cell will not be osmotically distended; the osmotic pressure of the contained liquid is nil; the suction pressure, the measure of its power to absorb or retain water, is zero. It will yield water, therefore, to the neighboring cells. The limiting conditions can be best understood by considering first a simplified case of one such dead cell in contact with one living cell. (We will suppose that they are protected from evaporation.)

Directly or indirectly, the pressure of the atmosphere acts upon them. The pressures are therefore

in the living cell, l:
osmotic pressure of cell sap, P_1;
hydrostatic pressure of cell sap, H_1, which balances the sum of the

wall pressure, W_1, and
atmospheric pressure, A.

in the dead cell, d:

osmotic pressure of the liquid, $P_d = 0$
hydrostatic pressure, $H_d = A$, since
wall pressure, $W_d = 0$.

The pressure determining the passage of water from the dead into the living cell will be the difference between $P_1 - H_1 = P_1 - W_1 - A$ and $P_d - H_d = 0 - A$, *i.e.* $P_1 - W_1$, which is the suction pressure of the living cell, as ordinarily defined (S_1).

Water will be withdrawn, therefore, till $W_1 = P_1$, *i.e.* till the living cell is fully turgid, unless resistance is offered to the consequent diminution of its volume by the wall of the dead cell. In the latter case the diminishing volume of water pulls upon the walls. Strictly speaking, the first effect is a reduction of the hydrostatic pressure of the water below atmospheric pressure, the water menisci are pushed back into the wall by the atmospheric pressure, an increasing share of which is, therefore, borne by the wall. If the wall were rigid it would come ultimately to support the whole of the atmospheric pressure acting on the outside of the cell. In the dead cell we should then have $H_d = 0$; $W_d = -A$.

The pressure determining movement of water would be the difference between $P_1 - W_1 - A$, and $P_d - H_d = 0$; *i.e.* $P_1 - W_1 - A$, or $S_1 - A$.

If the suction pressure of the living cell were greater than one atmosphere the removal of water from the dead cell would continue, increasing the strain on the wall and bringing the water in it into a state of tension, so that $W_d = -A + H_d$ where H_d is negative. Call the cohesion tension $C_d = -H_d$.

For equilibrium now between living and dead cells $P_1 - H_1 = P_d - H_d$, *i.e.* $P_1 - W_1 - A = 0 - H_d = C_d$, or $S_1 = C_d + A$.

The significance of this is, that if a cell loses protoplasm and solutes it will retain its water and resist collapse only if its wall is capable of withstanding a pressure difference, acting inwards, equivalent to the suction pressure of the neighboring cells. Actually, this pressure, up to the value of an atmosphere, is the external pressure of the atmosphere unbalanced by an equivalent hydrostatic pressure within the cell; beyond one atmosphere it is atmospheric pressure acting outside together with a cohesion tension pulling on the inside.

If the dead cell contains, not water, but a solution, as at first it probably does, P_d is not zero and the equations require modification accordingly. An important difference between dead and living cells is then that the former (assuming the wall completely permeable to the solutes) will

not be turgid and the full osmotic pressure of its sap will be active, unbalanced by any turgor pressure. Equilibrium would require that $S_1 = P_d$.

Clearly, therefore, before the dead cell reaches a state of complete collapse the solutes must be completely removed.

Applying these considerations to the contractile root of *Brodiaea lactea*, we conclude that the progressive collapse of the yielding layers requires the progressive removal of their sap solutes. The withdrawal of water follows so long as the plant is not fully turgid. In so far as the tissues of the root are continuous and free from air-spaces, the pressure differences and, ultimately, cohesion tension will not act directly on the walls of the collapsing cells themselves but on the surfaces of the root (external or internal) in contact with the air. As DIXON has emphasized by reference to a model recently (**1**), a water deficit acts throughout the plant as if a reduced pressure or a tension were acting on all its surfaces, tending to compress the plant. Living cells withstand the pressure osmotically and their suction pressure is a measure of the compressing force they have to withstand. Dead cells, with permeable membranes allowing the escape of their solutes, or having yielded their solutes before death, can only avoid collapse in so far as their walls are rigid enough to withstand the compression or are protected by other structures taking the mechanical strain. In the case under consideration, the periderm and the stele are such structures offering resistance to longitudinal compression; and in proportion as the walls of the collapsing cells are weak, these tissues are left to bear the brunt of the compressing force.

The limits to the actual magnitude of this force in any particular case will depend upon the water balance of the plant when contraction is taking place. There is no reason to doubt its adequacy to produce all the deformations observed, seeing that it has generally to be measured in atmospheres. Although the experimental difficulties would be considerable, it should be possible to measure the force of contraction directly; but so far it seems that no one has made the attempt.

Summary

It is suggested that the explanation already offered of the mechanism of the contractile roots of *Oxalis incarnata* is also applicable to those of *Brodiaea lactea*. In both, transverse zones of cells collapse, and there is no tissue showing the continuity essential to an effective contractile tissue. The forces which bring about the ascent of sap are adequate to contract a root showing the features common to the roots of these two plants.

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LITERATURE CITED

1. DIXON, H. H. Über die Saugkraft. Ber. deutsch. bot. Ges. **48**: 428–432. 1930.
2. HÖFLER, KARL. Ein Schema für die osmotische Leistung der Pflanzenzelle. Ber. deutsch. bot. Ges. **38**: 288–298. 1920.
3. RENNER, O. Experimentelle Beiträge zur Kenntnis der Wasserbewegung. Flora **103**: 171–247. 1911.
4. RIMBACH, A. Die kontraktilen Wurzeln und ihre Thätigkeit. Fünfstücks Beitr. wiss. Bot. **2**: 1–28. 1898.
5. ————————. Ueber die Lebensweise des *Arum maculatum*. Ber. deutsch. bot. Ges. **15**: 178–182. 1897.
6. ————————. Lebensverhältnisse des *Allium ursinum*. Ber. deutsch. bot. Ges. **15**: 248–252. 1897.
7. SMITH, F. H. The corm and contractile roots of *Brodiaea lactea*. Amer. Jour. Bot. **17**: 916–927. 1930.
8. STILES, W. Permeability. London, 1924 (pp. 95–101).
9. THODAY, D. The contractile roots of *Oxalis incarnata*. Ann. Bot. **40**: 571–583. 1926.
10. ————————. On turgescence and the absorption of water by the cells of plants. New Phyt. **17**: 108–113. 1918.
11. URSPRUNG, A., and BLUM, G. Über die Verteilung des osmotischen Wertes in der Pflanze. Ber. deutsch. bot. Ges. **34**: 88–104. 1916.
12. ————————. Zur Methode der Saugkraftmessung. Ber. deutsch. bot. Ges. **34**: 525–539. 1916.
13. DE VRIES, H. Ueber die Kontraktion der Wurzeln. Landw. Jahrb. **9**: 37–80. 1880.

BRIEF PAPERS

THE SENSITIVITY OF RED CLOVER (*TRIFOLIUM PRATENSE*) TO SMALL AMOUNTS OF BORON AND MANGANESE[1]

(WITH ONE FIGURE)

In these brief notes attention is drawn to the extreme sensitivity of red clover (*Trifolium pratense*) to the omission of small amounts of boron and manganese where the plants are grown in distilled water culture solutions.

Many experiments of this nature have already been carried out by several workers both in this country and abroad. In many cases however depression in growth through lack of boron has not been obtained until the chemicals involved in the culture solution have been carefully purified so

TABLE I

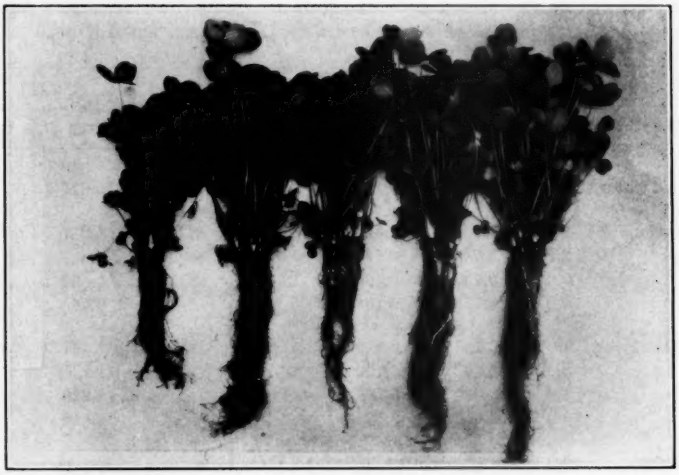
DRY WEIGHTS OF RED CLOVER CULTURES GROWN IN SOLUTIONS TO WHICH VARYING AMOUNTS OF BORON AND MANGANESE WERE ADDED

CULTURE SERIES		I		II		III	
TREATMENT		TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Distilled water alone		0.706	0.196	0.870	0.350	0.617	0.183
	Ppm.						
Boron,	1.0	0.726	0.203	0.976	0.346	0.937	0.313
	2.5	0.736	0.196	0.963	0.300	.....	.....
	5.0	0.736	0.200	1.010	0.336	0.620	0.187
	10.0	0.716	0.186	.....	.....	0.463	0.110
Manganese,	1.0	0.723	0.210	.....	.....	1.367	0.517
	2.5	0.733	0.190	.....	.....	.....	.....
	5.0	0.660	0.183	.....	.....	1.220	0.407
	10.0	0.663	0.186	.....	.....	0.880	0.290
Boron, 1.0 / Manganese, 1.0		0.763	0.206	.....	.....	1.407	0.550
Boron, 2.5 / Manganese, 2.5		0.756	0.193	.....	.....	.....	.....
Boron, 5.0 / Manganese, 5.0		0.630	0.173	.....	.....	0.917	0.247
Tap water		0.776	0.230	1.206	0.408	1.090	0.347

[1] Contribution no. 408 of the Rhode Island Agricultural Experiment Station.

as to be practically boron-free. No such precautions were taken with the cultures here reported.

While carrying out experiments to determine the nutrient requirements of redtop, Rhode Island bent grass, fescue and red clover, cultures were used with both tap and distilled water. With the grasses above mentioned, growth was uniformly good in both media. The growth of red clover, however, was distinctly depressed in the distilled water cultures. SOMMER and SOROKIN[2] have shown distinct morphological characteristics accompanying the lack of boron with *Pisum sativum*. As the gross root development was not normal the addition of small quantities of boron to the culture solution was indicated. Accompanying the depressed root development was a marked lighter color of leaves which might be considered as a form of incipient chlorosis. From previous work at this station and elsewhere a lack of manganese has been linked with chlorotic conditions. Boron was therefore added to the distilled water cultures in varying quantities, sodium



Distilled H_2O plus nutrient	1 ppm. boron plus nutrient	1 ppm. manganese plus nutrient	1 ppm. boron, 1 ppm. manganese plus nutrient	Tap H_2O plus nutrient

FIG. 1. Effect on the growth in culture solution of red clover (*Trifolium pratense*) of the addition of small amounts of boron and manganese.

[2] SOMMER, A. L., and SOROKIN, HELEN. Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. Plant Physiol. 3: 237–254. 1928.

borate being used. Manganese in the form of the sulphate was also supplied.

Table I gives the dry-weight yields of cultures with the various treatments used. The cultures were grown in triplicate and three series of cultures were grown at different times of the year. It will be noted that the yields of both tops and roots were increased by 1 part per million of boron; 2.5 parts per million of manganese, and a combination of boron 1 part per million and manganese 1 part per million. Greater amounts of these elements tended to depress growth. From figure 1 a visual comparison of the cultures may be obtained. It would therefore seem that the following two conclusions may be drawn.

1. When compared with certain grasses red clover, when grown in solution cultures, is much more sensitive to the absence of small amounts of boron and manganese.

2. Small amounts of boron and manganese when added to distilled water solutions tend to bring about increased root and top growth with red clover.—B. E. Gilbert and F. R. Pember, *Rhode Island Agricultural Experiment Station.*

OBSERVATIONS ON THE RED COLOR OF THE BLOOD ORANGE

(WITH ONE FIGURE)

Wheldale[1] makes the statement that anthocyanins are absent from citrus fruits "except in the red-fleshed variety, the so-called 'Blood Orange.'" She does not, however, give any experimental observations. The writer wishes to report the occurrence of the pigment in the fruit in crystalline form as well as to report some observations on the clear filtered juice.

The filtered juice has a pink to red color depending on the amount of pigment present. When acid (citric or hydrochloric) is added it turns to a deep pink or rose pink. The red pigment is not extracted from the juice by chloroform or ether. Ethyl acetate and amyl alcohol remove some of the red coloring matter. Sodium hydroxide changes the color to an emerald green. Lead carbonate and lead acetate produce a yellowish-green precipitate. The red color disappears when the juice is treated with zinc dust and hydrochloric acid but returns when the treated juice is allowed to stand.

Microscopic observation of the crushed juice sacs showed spherites or needle crystals of a deep red to reddish-brown color. One large group of crystals was blue-red, that is, about half way between a pure blue and a

[1] Wheldale, M. The anthocyanin pigments of plants, footnote 1, p. 27, Cambridge, 1916.

pure red. A typical group of crystals is shown in the accompanying photomicrograph (fig. 1). Globules of red solution were also present, probably

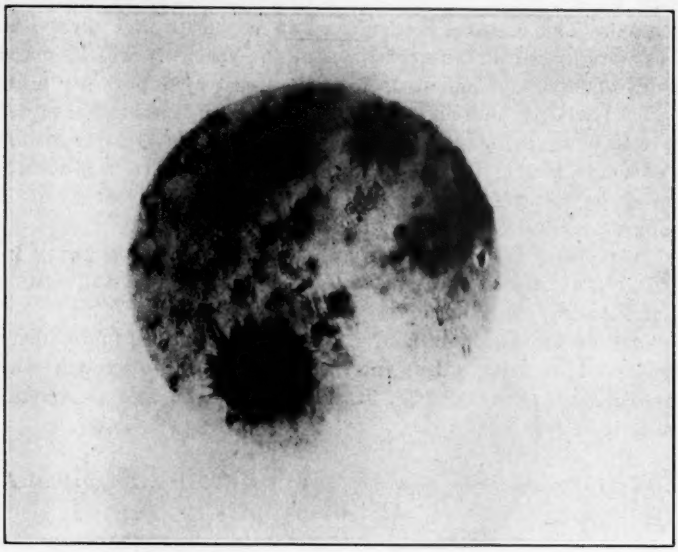


FIG. 1. Anthocyan crystals, ×110.

held in separate cells of the juice sac. In one case long red needle-like crystals were found adjacent to a lump of deep red material. The red globules of solution and the crystalline material became clear blue when a drop of ammonium hydroxide was allowed to flow under the coverslip.—M. B. MATLACK, *Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture.*

NOTES

Annual Meeting.—The annual meeting of the American Society of Plant Physiologists at New Orleans during the week of December 28, 1931–January 2, 1932, should be largely attended. Meetings in the far south are rare. Not since 1904 has New Orleans been host to the scientific associations of America. Only twice during this long period have meetings been held south of Washington, D. C.: Atlanta in 1913, and Nashville, 1927. At Atlanta the weather was uncomfortably cold during the entire meeting. The Nashville meeting was memorable for the fine weather during the meetings, and the blizzard on the way home to all points north. New Orleans should provide an ideal setting for winter-time meetings, and visitors to that city will find much of historical, architectural, and social interest in addition to the scientific value of the meetings.

Members will have opportunity to offer papers to the program committee at an early date. Papers to be read by title only are not desirable for such meetings, and authors offering papers are urged to present them in person. A prompt response to requests for titles is always appreciated by the committee in charge of the program.

The headquarters for the meetings of the American Society of Plant Physiologists are in the St. Charles Hotel, New Orleans. Early reservation by those who are planning to attend will prevent disappointment in securing accommodations.

Life Membership Committee.—President Tottingham has named the committee for the CHARLES REID BARNES life membership award, which is to be made at New Orleans. The personnel of the committee is as follows: Dr. R. H. CARR, Purdue University, chairman; Dr. B. E. GILBERT, Rhode Island Agr. Exp. Sta.; Dr. E. M. HARVEY, U. S. Dept. Agr., Pomona, Calif.; Dr. W. D. KIMBROUGH, University of Louisiana; Dr. W. J. CRIST, Michigan State College. The announcement of the award has usually been made at the banquet held during the annual meeting.

Reprints.—The policy of PLANT PHYSIOLOGY with reference to free reprints may need clarification or at least restatement. In order to obtain free reprints the author must place an order for reprints for which he expects to pay. Against this order the first costs of making them up are assessed. In addition to this order, we then have made up fifty reprints without covers and include them in the shipment with no additional charge. If for instance an author wants 150 reprints without covers, he can place his order for 100, and will receive 150. If he places an order for 150, he will receive 200, etc. The author should take into consideration the reprints

furnished free when ordering. No reprints are made up if the author places no order for them.

Costs of Publication.—Members of the Society will be interested in the three items of cost involved in the production and distribution of the official journal, PLANT PHYSIOLOGY. Intelligent interest in these costs might assist in bringing about more economical publication; at least, certain items of cost could be reduced if members took an active interest in these matters. The figures for the January, 1931, and July, 1931 issues are as follows:

	January	July
Printing (including covers, and corrections)	940.58	939.87
Engravings	170.27	138.97
Distribution costs (postage, wrapping, stencils)	39.91	43.65
Total	$1,150.76	$1,122.49

These figures do not include the cost of separates presented to the authors of papers, but otherwise represent the entire cost to the Society of an edition of 1,000 copies of each number.

Specific attention is directed to the matter of corrections, included in the bill for printing costs. These cost from $15.00 to $40.00 per issue. The loss from this item cannot be eliminated entirely, but it can be reduced. It results mainly from the practice of certain authors who insist on rewriting their papers in galley proof. This is of course an absolutely illegitimate practice. The oversupply of papers from which nearly all journals suffer would be reduced somewhat if authors who take these illegitimate liberties would hold their papers long enough to perfect them before submitting them. In linotype printing, change of a letter involves resetting a whole line. Insertion of a word or phrase may require resetting of several lines, or a whole paragraph. For these changes the treasurer must pay. As the thoughtlessness of authors toward the problems of publication is so wide spread, we solicit earnest cooperation to stop the leaks in our printing costs.

Size of Manuscripts.—PLANT PHYSIOLOGY since March 1931 has had a definite surplus of publishable material. The editorial board has been averse to setting a size limit to papers offered for publication, but it is just inevitable that some policy must be adopted to control the size of the annual volumes of the journal. The American Society of Plant Physiologists is giving to its members and subscribers more literature per year than almost any other society of its age has ever offered. For example, no volume of the American Journal of Botany before 1929, the 16th volume of that journal, has contained as many pages as this, our 6th volume. Similar comparisons can be made with other journals, Phytopathology, Ecology, Jour-

nal of the American Society of Agronomy, etc. A very generous amount of space has been used, and yet it is impossible to publish acceptable manuscripts as fast as they are received.

To meet the situation a tentative method of control is being adopted which is intended first of all to discourage long papers, and secondly to control the size of the annual volume. We hope it will meet the approval of the general membership, and the editor would like an expression from a wide circle, either of approval or disapproval. The size of papers will not be limited arbitrarily, although size alone may determine the rejection of otherwise suitable papers. But the *number* of long papers (*i.e.*, exceeding 30 printed pages) used in each volume must be strictly limited for each volume. By experience in 1931 it has been found impossible to use more than three long papers during the year. The tentative proposal, therefore, is to include not more than one long paper in each of the first three issues of the year, and to make up the final number solely of shorter papers, and to limit the size of the volume to fit the income of the Society from memberships and subscriptions. The lengthy papers will be placed in a separate series from the shorter ones, and used in chronological order. The editor now has on hand about six of these long papers. Authors should guide themselves accordingly. Scrutiny of long papers reveals the fact that some of them could have been improved considerably by condensation. Comment on this proposed plan is desired by the editorial board, and will be gratefully considered.

Errata.—A number of errors have been detected in the pages of PLANT PHYSIOLOGY after publication. These have been listed at the close of the table of contents. We suggest that these errors be corrected at the places where they occur, to prevent future misinterpretation. The editor is grateful to the authors who assisted in preparing the list, and such cooperation is invited on the part of anyone finding mistakes in the journal.

Portraits.—A complete set of the 13 portraits published in PLANT PHYSIOLOGY during the last several years may be obtained for $1.45, postpaid. In broken lots, send 12 cents in stamps for each portrait desired. They are suitable for offices, corridors, laboratories, libraries, etc., and stimulate interest in the historical development of science, particularly of plant physiology. Orders will receive prompt attention. Address the editor of PLANT PHYSIOLOGY, University of Chicago.

Principles of Plant Biochemistry.—This work is a publication from the University of Cambridge Press by Mrs. MURIEL WHELDALE ONSLOW, whose "Practical Plant Biochemistry," and "Anthocyanin Pigments of

Plants'' are well known among plant physiologists. The book is devoted particularly to the chemistry of the carbohydrates and proteins, and to respiration. The two carbohydrate chapters consider the family of sugars, and the cell wall and its modifications. The third chapter takes up oxidizing and reducing systems, the fourth and fifth chapters deal with proteins of plants and nitrogen metabolism, and the final chapter with respiration. Eleven literature lists accompany the main sections, and provide about 975 references. While these do not properly reflect the American contributions to plant biochemistry, they offer a good summary of progress made in Europe. The book is obtainable for $4.75, from the Macmillan Co., New York.

Alkaloids.—The second edition of G. TRIER's great monograph, Die Alkaloide, has now been completed. The second volume has appeared this summer, the first having been printed in 1927. The volumes are paged consecutively, and contain 1,061 pages. The first 860 pages (Spezieller Teil) are occupied by accounts of I. the better known alkaloids, arranged in groups, aliphatic bases, aromatic bases, acid amides, urea derivatives, and heterocyclic bases of various types; II. the less well known bases found in cryptogams and phanerogams, arranged by families in the Engler-Prantl order; and III. chemically little known bases of general distribution (ptomaines, vitamines, hormones, etc.). The second section, pp. 861–984 (Allgemeiner Teil) deals with the problems of constitution and distribution of alkaloids, their biochemical and pharmacological significance, analysis and determination of constitution, etc. There is a supplement (Nachtrage), of 38 pages at the rear, a concluding discussion of tryptophane and the hypothesis of alkaloidal origin of blood- and leaf-pigments, an alphabetic list of the alkaloid-containing plant families (phanerogams only) and the index. The price, bound, is listed at 42 RM. Anyone desiring to order copies of this important monograph for personal or library use should have orders sent to Gebrüder Borntraeger, Schöneberger Ufer 12a, Berlin W 35, Germany.

Fruit Culture.—The trend of interest in scientific plant production the world over is toward a physiological foundation for cultural practice. A new text-book emphasizes this trend. It is FRITZ KOBEL's Lehrbuch des Obstbaus auf physiologischer Grundlage, from the press of J. Springer, Berlin. This interesting book presents first the general physiology of fruit trees, intake of water, transpiration and water transport, absorption and utilization of mineral nutrients, photosynthesis, storage and use of reserves, effects of low and high temperatures, and vegetative growth.

The second section deals with blossom bud differentiation and growth, and the ways in which many environmental factors affect these processes.

Then the processes of fruit formation are considered, beginning with the opening of the flower, fertilization, and the normal fruit development following this process, etc. Such problems as sterility receive much attention, parthenocarpy and apogamy are treated briefly, and the development of the fruit up to the ripe stage is depicted as a dynamic physiological process.

The last two sections deal with the relations between vegetative growth, fruit bud differentiation, and fruiting behavior, and with fruit breeding for new varieties. The book frankly accepts the carbohydrate-nitrogen relation hypothesis as the basis of explanation of much of the physiological behavior of fruit trees. It contains 274 pages of text, and is written in a style that will please those who are beginning to read and appreciate German scientific literature. It is recommended not only for its readability, but also for its solid value as a contribution toward physiological perspective in the plant sciences. The price is 16 RM. for unbound copies, or 18.4 RM. for cloth binding. Orders may be sent directly to Julius Springer, Linkstrasse 23–24, Berlin W 9, Germany.

Physical Properties of the Soil.—A summary of the available knowledge concerning the physical propertites of the soil has been prepared by Dr. B. A. KEEN, soil physicist of the Rothamsted Experimental Station. It is one of the Longmans, Green and Co. series of Rothamsted Monographs on Agricultural Science. The opening chapter is an historical introduction presenting the relations between the development of knowledge of soil physics and the development of the arts and implements of soil culture. Chapter II considers the methods of physical soil analysis, and chapter III the physics of distribution and water movement in the soil. Succeeding chapters discuss the properties of soils of low moisture content, soil and clay pastes, soil and clay suspensions, soil constants and equilibrium points, and physical properties of soils under field conditions. The final chapters deal with soil temperature and soil aeration. The literature of soil physics is cited freely, and the bibliography at the close cites more than 250 papers, ample justice being done to contributions from this side of the Atlantic. Many footnotes cite other pertinent papers. The price of the book is $8.00, and copies may be obtained from Longmans, Green and Co., New York.

Chemical Plant Physiology.—Dr. S. KOSTYCHEV's Chemische Physiologie der Pflanzen, which was published in a German edition in 1926, has been translated by Dr. C. J. LYON, of Dartmouth College. The value of a translation is usually determined by the accuracy of the translator, and the faithfulness with which the original work has been followed. In this case, however, KOSTYCHEV rewrote parts of the original especially

for this translated edition, and many changes will be found by one who compares the two. The chapter headings remain the same, and no changes have been made in the order of presentation. Editorial notes will be found among the foot-notes, with citations of literature pertinent to the discussion. Since many American students still do not make the effort to read German fluently, the translation will be helpful and time-saving. On general principles we do not believe in the translation vogue, and would prefer to see the originals in use by American students. The price of the work is $6.00, and the publishers are P. Blakiston's Son and Co., Philadelphia.

Soil and Microbe.—An attractive account of the microbiology of soils has been written by Dr. S. A. WAKSMAN and R. L. STARKEY, of Rutgers University. It is entitled "The Soil and the Microbe." There are ten chapters, the first of which is concerned mainly with the processes of soil formation and plant nutrients. Chapters II and III discuss the microbes of the soil, their distribution and activities, and chapter IV the microbic decomposition of organic substances in the soil. The next three chapters deal with the transformations of soil nitrogen and mineral substances through the direct or indirect activity of microorganisms. Chapter VIII takes up the interrelations of higher plants and soil organisms, root excretions, mycorrhizas, nodule formation by legumes, bacteriorrhizas, etc. Chapter IX considers the modification of soil populations by cultural practices, and the last chapter summarizes the importance of microbes in soil fertility. A fairly comprehensive view of this field is presented, and the fundamental principles are set forth very clearly. The book is well illustrated, and many tables of data are included to elucidate the discussion. Each chapter closes with a small bibliography, but without periodic literature citations. The price of this useful book is $3.50, and copies may be secured from the publishers, John Wiley and Sons, New York.

Handbook of Plant Nutrition.—The second volume of this work was noticed in the April, 1931 number of PLANT PHYSIOLOGY. We have now received the first volume, Pflanzenernährung, and it is a worthy companion to the preceding one. It contains 945 pages, and the ten chapters have the following titles: History of the development of plant nutrition; constituents and composition of the plant body; the cycle of substances in nature; the physiology of metabolism of plants; the soil as the habitat and nutrient reservoir for plants; the law of yield; water culture and growth experiments; field experiments; evaluation of fertilizer experiments; and determination of the nutritive deficiencies of the soil. Both volumes are well illustrated, and carry many literature citations at the ends of the various sections. It impresses one as a work of great value for scientific

workers, and for agriculturists who are interested in scientific plant culture. The editor of the volumes, Dr. F. HONCAMP, of Rostock, and the publishers, J. Springer, Linkstrasse No. 23–24, Berlin W 9, deserve the congratulations and thanks of all plant science investigators for this great contribution to plant nutrition and fertilizer science literature. The price of volume I unbound is RM. 93, bound RM. 96.8; and of volume II unbound is RM. 86, bound RM. 89.8. Orders may be sent directly to the publishers, in Berlin.

Plant Analysis.—The first volume of a four-volume Handbuch der Pflanzenanalyse has been published by J. Springer, I. Schottengasse 4, Vienna. The author, or editor, is G. KLEIN, each section being prepared by someone familiar with that particular phase of analytical work. This volume is mainly given over to general chemical and physical methods, rather than to systematic examination of plant tissues for their constituents. Such matters as the testing of reagents for purity, weighing, heating and cooling of materials, drying, recrystallization, filtering, centrifuging, dialysis, solubility separations, distillation and sublimation, occupy the first several chapters. Determination of elements, detection of radicals and groups by their characteristic reactions, gravimetric and volumetric procedures, histochemical methods, specific gravity, melting and boiling points, solubility, viscosity, and molecular weight determinations follow. Then come chapters which give methods for optical examination of materials, polarization, refractometry, interferometry, spectroscopy, spectrophotometry, colorimetry, etc., fluorometry, fluorescence, ultramicroscopy, and photochemical analysis. Other sections deal with electrical conductivity, electrometric and colorimetric determinations of hydrogen-ion concentration, and colorimetry. The closing sections deal with general procedures in chemical analyses, which will be continued in the second volume.

It is evident that the work covers a very wide range of methods, and that it will be a useful handbook for everyone who has to examine plant materials in any way whatever. The first volume contains 627 pages, and 323 figures. The price quoted for this volume is RM. 66 in paper cover, or RM. 69 bound in cloth. Orders for the complete work can be filed with the publisher, but the cost of the three unpublished volumes has not been stated. Libraries and research laboratories will find the work an indispensable aid in analytical work of all kinds.

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